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EXPERIMENTAL TRANSMISSION OF THE MOSAIC OF ABACÁ, OR MANILA HEMP PLANT (MUSA TEXTILIS NÉE)[1]

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WITH FIVE PLATES

The mosaic of abacá, or Manila hemp plant (*Musa textilis* Née), is a destructive disease. Its occurrence in Davao, Mindanao, was reported by Calinisan in 1934 and by Ocfemia in 1937. In addition to abacá, Ocfemia found that a wild *Canna indica* Linn. is attacked by a disease which causes mottling of the leaves similar to that of abacá mosaic.

Calinisan (1934) attempted to transmit mosaic of abacá with the use of *Pentalonia nigronervosa* Coq. as vector but failed to secure transmission of the disease. Owing to the fact that Magee (1930) suspected that *P. nigronervosa* is the carrier of the mosaic of banana in Australia, Calinisan (1938, p. 312) concluded that "... *P. nigronervosa* seems to have some relation with the transmission of the abacá mosaic." Ocfemia and Celino (1938) could not transmit abacá mosaic with *Pentalonia nigronervosa* but they showed that the disease is readily transmitted by *Aphis gossypii* Glover and by two other species of aphids. These authors also showed that abacá mosaic may be transmitted by *Aphis gossypii* to the wild *Canna indica*. The transmission of the abacá mosaic virus to *Canna indica* suggests that the abacá disease may be related to the mosaic of *Canna indica*. Kunkel in 1928 listed *Canna indica* Linn. as one of the plants susceptible to mosaic.

As the mosaic is spreading in Davao with increasing severity, the need for more definite information regarding the disease stimulated the present investigation.

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[1] Thesis presented in partial fulfillment of the requirements for the degree of Master of Science in Plant Pathology, University of the Philippines. Experiment Station contribution No. 1378. Prepared in the Department of Plant Pathology under the direction of Associate Professor G. O. Ocfemia.

THE DISEASE

Distribution and economic importance

At present the mosaic of abacá seems to be confined to Davao. The disease attacks abacá either independently of bunchy-top or in combination with it. In spite of the attempts of the abacá planters in Davao to control the disease, it has spread in alarming proportions. In his visit to the College of Agriculture in August, 1937, and on April 30, 1940, Mr. U. Kojima, of the Ohta Development Co., Inc., stated that in the eastern part of Davao about 50 per cent of the abacá plants were infected. Owing to the destructiveness of the disease and to the rapidity that it spreads, mosaic is regarded as one of the three major diseases of abacá in Davao. The disease is not known in Luzon and other parts of the Philippines where abacá is grown.

Symptoms

Calinisan (1939) described in detail the advanced symptoms and effects of mosaic on the leaves, pseudostems, flowers, and fruit of abacá. Owing to his failure to transmit abacá mosaic with the use of insect vectors, this author could not describe the earliest symptoms of the disease. As it is most important to know the first symptoms of infection with any disease because they are essential in an eradication campaign, disease survey, and extension work, the writer gave particular attention to the first visible indications of mosaic infection. The writer agrees with Calinisan (1939, p. 121-122) that there is no need for describing in detail the symptoms of abacá mosaic because they are very characteristic and a mosaicked abacá cannot be confused with another disease. Calinisan (1939) further states that any person who has a normal vision can readily recognize an infected plant, at some distance, by the mottling of the leaves. The mottling of the leaves, petioles, and leaf sheaths of abacá seedlings a few months old does not differ much from that of mature plants.

The first symptoms of infection can be studied only in transmission experiments conducted under controlled conditions. Ocfemia described the first indications of bunchy-top of abacá (1930) and of Fiji disease of sugar cane (1934) on plants infected in insect-transmission experiments. In aphid-transmission experiments of abacá mosaic, the writer noted that the first symptom of infection appears eight to ten days after the insect carriers have been allowed to feed on healthy abacá seedlings. The first symptom consists of the presence of small, pale white dots, not larger than the head of

a pin, on the newly expanded leaf (Pl. 1). These minute, more or less circular, pale areas gradually elongate into short, narrow dashes but the central dot-like beginnings remain distinct. Later, more definite and clearer symptoms appear on the next leaf. Light yellowish patches about three to five millimeters wide and twenty or thirty millimeters long appear on the lamina of this leaf. The mottled appearance is shown later on the succeeding leaves (Pl. 2). In advanced stages of infection the mottling appears also on the petioles of the leaves and on the pseudostems of succeeding leaves. As the leaves mature the mottling becomes more or less diffuse and less distinct. The mottled areas gradually turn brown to reddish brown and eventually dry out. Mosaic does not cause pronounced bunching of the leaves. Neither does it produce colorless or transparent veins in the leaves similar to those caused by bunchy-top, though Calinisan (1939, p. 124) states that the ... "characteristic incipient symptom may be noted simultaneously with the clearing of the veins in between them (spindle-shaped patterns) ...".

As the mosaic disease affects the chloroplasts, the production of food by the leaves must necessarily be affected. Owing perhaps to insufficiency of food, mosaicked plants are more or less stunted in growth and they produce slender pseudostems of little or no commercial value. As a final stage of the disease, the roots are weakened and are readily invaded by fungi and bacteria in the soil.

CAUSE OF THE DISEASE AND METHODS OF TRANSMISSION

The mottling of the leaves, petioles, and pseudostems of abacá is a characteristic of mosaic diseases. Owing to the fact that mosaic diseases are caused by virus the writer determined the various ways that the abacá disease may be spread from plant to plant.

According to Magee (1940) the banana mosaic, or infectious chlorosis of banana in Australia is caused by a virus identical with *Cucumis Virus I.* He also states that an identical virus causes, on *Musa sapientum* Linn., symptoms which Wellman described and attributed to *Celery Virus I.* In accordance with Holmes' nomenclature of the viruses, the banana mosaic, or infectious-chlorosis virus is *Marmor cucumeris* Holmes (1939).[2]

Magee (1940) reports several weed hosts of the banana mosaic virus in Australia. This author believes that outbreaks of mosaic

[2] The Latin binomials used in this paper are based on the system of virus nomenclature in HOLMES, F. O. 1939. Handbook of phytopathogenic viruses. v + 221 p. Minneapolis, Minn.: Burgess Publishing Company.

are likely to appear where vegetables such as cucurbits and tomatoes are grown close to banana fields. In addition to *Pentalonia nigronervosa* Coq., Magee (1940) reports that the virus of the banana mosaic may be communicated by *Macrosiphum gei* Koch, *Aphis gossypii* and an unidentified species of *Aphis*.

In 1938, Ocfemia and Celino transmitted abacá mosaic to abacá and *Canna indica* with the use of *Aphis gossypii* Glover and of two other species of aphids.

As most virus diseases are not transmitted through seeds, all abacá seedlings used in transmission experiments were grown from seeds. The seeds were germinated in sterile soil; the seedlings were pricked and later potted according to the method described by Ocfemia in 1930. The method of transferring the aphids from sources of inoculum to the experimental abacá plants and the subsequent treatments of the plants in all insect-transmission experiments and in transmission by various mechanical means were also in accordance with those described by Ocfemia (1930).

Transmission experiments using Pentalonia nigronervosa Coq.[3]

Experiment 1. On July 20, 1937, five healthy, six month old abacá seedlings were used in transmission experiments. One-hundred individuals of *Pentalonia nigronervosa* Coq. which were previously reared on mosaicked abacá were transferred to each seedling. Healthy abacá seedlings to which aphids from healthy abacá were transferred served as control.

The aphids were allowed to feed on the experimental and on the control plants for ten days and then the plants were sprayed with dilute soap solution to kill the aphids. The plants were placed on wooden benches (Ocfemia, 1930, Pl. I, C) outside of the laboratory for observation.

Experiment 2. With the method described in Experiment 1, five abacá seedlings were used for colonizing *Pentalonia nigronervosa* taken from diseased abacá on July 24, 1937; four plants on July 26, 1937; three plants on December 12, 1937; five plants on June 8, 1938; two plants on June 17, 1938; three plants on August 20, 1938; three plants on August 23, 1938; five plants on August 24, 1938; four plants on September 5, 1938; and five plants on November 10, 1938.

On September 1, 1939, when the final examination of the plants used in transmission experiments was made, it was noted that *Penta-*

[3] All insects used in transmission experiments in this paper have been determined for the writer by the Department of Entomology, College of Agriculture.

lonia nigronervosa cannot transmit abacá mosaic. According to Calinisan (1938, p. 311-312), *Pentalonia nigronervosa* seems to have some relation to the transmission of abacá mosaic because the healthy abacá seedlings which he planted side by side with mosaicked abacá developed mosaic and the infected plants were heavily infested with *Pentalonia nigronervosa.*

In 1930, Magee (1940) showed that the banana mosaic in Australia may be transmitted by *Pentalonia nigronervosa.* As the transmission of the banana mosaic by *Pentalonia nigronervosa* has been confirmed by Magee (1940) only with difficulty, he regards this aphid as an unsteady vector and for that reason it is probably of no importance in the field.

Although *P. nigronervosa* is the vector of bunchy-top of abacá in the Philippines (Ocfemia, 1926, 1930) and of bunchy-top of banana in Australia (Goddard, Magee, and Collard, 1926) the writer conclusively proved that it cannot transmit mosaic of abacá.

Transmission experiments using Rhopalosiphum nympheae (Linné)[4]

Experiment 1. On April 30, 1938, fifty individuals of *Rhopalosiphum nympheae* (Linné) from mosaicked abacá (Pl. 3, fig. 1) were transferred to a six-month old abacá seedling.

On May 17, 1938, eighteen days after the aphids were allowed to feed on the experimental abacá, a definite symptom of mosaic was observed on the unfurled leaf of the plant.

Experiment 2. On May 17, 1938, fifty adult aphids were transferred to each of three abacá seedlings. On May 26, 1938, four abacá plants were added and on July 22, 1938, five other seedlings were used. Control seedlings were provided in each case.

Although fewer than *P. nigronervosa* were used in these two experiments, all of the abacá seedlings on which *Rhopalosiphum nympheae* were allowed to feed became infected with mosaic in less than a month. The periods of incubation of the disease ranged from eight to twenty-three days. All check plants remained healthy throughout.

Transmission experiments using Aphis gossypii Glover

Experiment 1. From August 22, to September 6, 1938, adults of *Aphis gossypii* Glover (Pl. 3, fig. 2) from mosaicked abacá were

[4] According to Dean L. B. Uichanco the brown aphid is apparently *Rhopalosiphum nympheae* (Linné).

placed on seventeen healthy abacá seedlings. Four other abacá seedlings to which aphids from healthy abacá plants were transferred and allowed to feed served as control. In less than two weeks, seven of the experimental plants showed early symptoms of mosaic and in about a month all of the seventeen abacá seedlings were infected with the disease. The periods of incubation of the disease ranged from thirteen to twenty-five days. None of the control plants showed mosaic.

Experiment 2. To each of twelve healthy abacá seedlings, fifty *Aphis gossypii* were transferred from mosaicked abacá. In twelve to twenty-eight days, one hundred per cent of the plants were infected.

Transmission experiments with Stephanitis typica (Distant)

Experiment 1. Large numbers of adults and nymphs of *Stephanitis typica* (Distant) were transferred from healthy banana to mosaicked abacá plants which were growing in insect-proof cages (Ocfemia, 1934, Pl. I; fig. 1 [top]). After allowing the insects to feed on the diseased plants for one week, twenty-four healthy, seven month old abacá seedlings were placed at different times inside the cages with the mosaicked plants. The healthy abacá seedlings were exposed to the insects inside the cages for periods which ranged from two to three weeks. Within these periods the insects fed at will from the diseased to the experimental abacá seedlings. Four healthy abacá seedlings in another wire cage to which 100 individuals of *S. typica* taken from healthy abacá plants were transferred served as control. Although the plants were kept under observation for twelve to thirteen months none of them developed symptoms of mosaic infection.

Experiment 2. Large numbers of nymphs of *S. typica* were reared to the adult stage on mosaicked abacá and then the insects were transferred to twelve abacá seedlings. The insects were allowed sufficient time to feed on the experimental plants. Even at the end of one year of incubation, however, none of the experimental plants became mosaicked.

The results of the experiments show that *Stephanitis typica* cannot transmit the virus of abacá mosaic.

Transmission experiments with Ferrisia virgata (Cockerell)

Experiment 1. In the summer of 1939, numerous *Ferrisia virgata* were found on the nether surface of the leaves of mosaicked and

healthy abacá plants outside of the laboratory of the Department of Plant Pathology. Fifty nymphs of the insect were transferred from mosaicked abacá to each of fourteen healthy, seven month old abacá seedlings. Three other plants, each with fifty nymphs taken from healthy abacá served as control. The experimental and control plants were bagged for ten days but the insects were allowed to remain on the plants for two to three weeks more after the bags had been removed and the plants were kept outside of the laboratory. Although the plants were under observation for twelve months none of them became diseased.

The results of insect-transmission experiments show that *Pentalonia nigronervosa, Stephanitis typica,* and *Ferrisia virgata* cannot transmit abacá mosaic. *Aphis gossypii* and *Rhopalosiphum nympheae* transmit the disease readily. The results further show that *Pentalonia nigronervosa, Stephanitis typica,* and *Ferrisia virgata* bear to the abacá mosaic virus the same relation that *Aphis sacchari* Zehntner, the woolly aphis, *Ceratovacuna lanigera* Zehntner, and the pink mealy bug, *Trionymus sacchari* (Cockerell), have to the virus of the mosaic of sugar cane, *Marmor sacchari* Holmes. Similarly, *A. gossypii* and *Rhopalosiphum nympheae* bear to the abacá mosaic virus the same relation that *Aphis maidis* Fitch, *Toxoptera graminum* Rondani, *Carolinaia cyperi* Ainslie, and *Hysteroneura setariae* Thomas (Ingram and Summers, 1936, 1938) have to the sugar cane mosaic virus.

Transmission of abacá mosaic by various mechanical methods

Experiment 1: *Needle-pricks.* With the method of inoculation described by Sein (1930) for sugar cane mosaic, healthy abacá seedlings were inoculated by pricking with a fine pointed needle mounted on a piece of bamboo rod. Three abacá seedlings were pricked on October 1, and three on October 9, 1937; four on July 15, 1938, and ten on June 1 and 5, 1939. In each case two other abacá seedlings were used as control. Even after a year of incubation, however, none of the plants which were inoculated on October 1 and 9, 1937, and on July 15, 1938, developed mosaic disease. The plants which were inoculated on June 1 and 5, 1939, did not show symptoms of infection either, although they were examined on March 30, 1940.

The inoculation by the needle-prick method was repeated on July and August, 1939, with twelve abacá seedlings. Until June, 1940, however, none of the plants became mosaicked.

Experiment 2: *Hypodermic syringe.* From October to December, 1937, twenty-four healthy abacá seedlings were inoculated by injecting into each seedling, one milliliter of freshly extracted and undiluted juice of mosaicked abacá. The juice was obtained from crushed leaves and petioles of diseased plants. In each experiment an equal number of plants was injected with juice of healthy plants to serve as control. After injection, the plants were placed on benches outside of the laboratory for observation. None of the plants injected with the virus extract developed mosaic.

Experiment 3: *Leaf contact.* One potted healthy abacá and one potted mosaicked plant of practically the same height and free from aphids were placed on a table. The young expanded leaf of each plant was drawn together, and the midribs were held against each other at a certain point with a paper clip. A sharp knife was inserted between the tightly held midribs and a slight cut was made on the surfaces of both midribs. The injured surfaces of the midribs were immediately held against each other by means of several paper clips. The two leaves were kept in this position for twenty to thirty days. The controls were similarly treated except that the plants used were both healthy. Five seedlings were used on October 3, 1938, six plants on October 5, 1938, and five plants on October 31, 1938. Only one of the plants showed infection with mosaic after an incubation period of twenty-eight days. Owing to the low percentage of infection it was believed that the plant already had the incipient stage of the disease when it was used in the transmission experiment.

The failure to transmit mosaic of abacá by various mechanical methods corroborates the results of Magee (1940) who states that he could not transmit by mechanical inoculation the banana mosaic, or infectious chlorosis of banana in Australia.

THE PHYSIOLOGY OF INSECT-TRANSMISSION

The smallest number of Rhopalosiphum nympheae necessary to inject virus and cause infection

Experiment 1: *Ten adult aphids.* Ten adult *Rhopalosiphum nympheae* taken from mosaicked abacá were placed on each of ten abacá seedlings from June 27 to September 7, 1938. Five seedlings on which virus free aphids were allowed to feed served as control. Of these ten seedlings five developed symptoms of mosaic and five did not. The period of incubation ranged from 26 to 43 days. The control seedlings did not develop mosaic.

Experiment 2: *Eight aphids.* From August 5 to September 21, 1938, eight adult aphids from mosaicked abacá were transferred to each of twenty-one abacá seedlings. Of these plants only two showed mosaic infection after incubation periods of 57 and 60 days, respectively.

Experiment 3: *Five aphids.* From September 15 to October 28, 1938, five adult aphids from mosaicked abacá were transferred to each of 24 abacá seedlings. None of the plants was infected even after the lapse of one year.

Experiment 4: *Three aphids.* From September 26 to October 3, 1938, three adult aphids were transferred to each of 24 seedlings. None of the plants became mosaicked even after prolonged incubation.

The results of using a gradually decreasing number of *Rhopalosiphum nympheae* show that at least eight aphids are necessary to introduce into a young abacá seedling enough virus to make an infective dose. Ocfemia (1930, p. 9) found that five adult *Pentalonia nigronervosa* were capable of transmitting bunchy-top to six out of nine abacá seedlings which were two months and twenty-two days old. He noted that the symptoms appeared after twenty days of incubation. In his experiments the writer noted that the greater the number of virus-bearing aphids that were allowed to feed on abacá plants the higher was the percentage of infection, and the period of incubation was shorter. This was expected because with a large number of aphids, more virus was introduced into the experimental plants and, consequently, the chances of producing infection were greater.

Transmission of virus from infective Rhopalosiphum nympheae to their offspring

The failure of the abacá mosaic virus to infect *Canna edulis* afforded a suitable plant on which to rear aphids to determine whether or not the virus is transmitted to the offspring of infective mothers.

Experiment 1. Large numbers of *Rhopalosiphum nympheae* were transferred from mosaicked abacá to healthy shoots of *Canna edulis*. At intervals of two to three hours, the young that were born were carefully picked up with the fine point of a camel's hair brush and transferred to shoots of healthy abacá seedlings. The young aphids remained on the *Canna* shoots for only a short time and had very little chance to feed on this host. Twelve abacá seedlings were used from October 1 to October 29, 1938, and fifty young aphids were

transferred to each seedling. To protect the aphids from insect predators and other enemies, the abacá seedlings were bagged, and the aphids were allowed to reach their adult stage on the plants. In a month the aphids on each plant increased in number considerably. In spite of this, however, mosaic did not develop on any of the experimental plants.

Experiment 2. The experiment was repeated using twelve abacá seedlings. As in the first experiment, however, none of the plants became diseased. The results of these two experiments show that the young born of infective *Rhopalosiphum nympheae* mothers did not carry the virus. Ocfemia and Buhay (1934) found that the virus of bunchy-top of abacá (*Marmor abacá* Holmes) is not transmitted from the parent *Pentalonia nigronervosa* to its progeny. These authors added that the importance of this in rehabilitation of completely devastated abacá fields is that it assures us that when the virus-bearing aphids die, their harm ends as there is no fear of their offspring transmitting bunchy-top. The young *Pentalonia nigronervosa* are infected only after feeding on volunteer abacá sprouts from infected corms (Ocfemia and Buhay, 1934).

With rare exceptions, insect carriers of virus diseases do not transmit the virus to the offspring. *Myzus fragaefolii* Cockerell does not transmit the strawberry crinkle disease virus (*Marmor fragariae* Holmes) to its offspring (Vaughan, 1933). The same is true of *Macrosiphum solanifolii* (Hoggan, 1933) and other aphid carriers. Ocfemia and Celino (1939) report that the nymphs of *Perkinsiella vastatrix* Breddin which hatch from eggs of virus-bearing adults are free from the Fiji-disease virus, *Galla fijiensis* Holmes (1939). Severin (1921) showed that the beet leafhoppers (*Eutettix tenellus* Baker) from eggs of infective adults were non-infective. *Nephotettix apicallis* Motsch. seems to be the only example of a virus carrier which transmits the virus of rice-dwarf disease (*Marmor oryzae* Holmes) to the young through the eggs (Fukushi, 1933).

The shortest time required by Rhopalosiphum nympheae to feed on mosaicked abacá in order to obtain the virus

Experiment 1: Forty-eight hours of feeding. Numerous virus-free *Rhopalosiphum nympheae* were allowed to feed on mosaicked abacá for forty-eight hours, and fifty aphids were transferred to each of ten abacá seedlings from August 3 to September 1, 1938. The abacá seedlings were bagged and the aphids were allowed to feed on these

seedlings for fifteen days. After a period of incubation ranging from nine to sixteen days seven of the seedlings showed symptoms of mosaic, but three did not.

Experiment 2: *Twenty-four hours of feeding.* From August 16 to September 17, 1938, adults of *Rhopalosiphum nympheae* were allowed to feed on mosaicked abacá for twenty-four hours, and fifty of them were transferred to each of ten experimental plants. In this experiment five developed the disease after periods of incubation ranging from ten to fifteen days.

Experiment 3: *Twelve hours of feeding and less.* In view of the readiness of infection when adults of *Rhopalosiphum nympheae* were allowed to feed for twenty-four hours, experiments were conducted with decreasing feeding periods as follows: 12, 6, 3, 2 hours, and 1 hour. In the lot where the aphids were allowed to feed on mosaicked abacá for twelve hours, five of the ten experimental plants developed mosaic after a period of incubation of thirteen to twenty-five days. In the six-hour feeding period, three of the ten experimental plants showed mosaic after a period of incubation of fourteen to seventeen days. In the three-hour feeding period, four out of ten plants became infected after an incubation period ranging from thirteen to twenty-one days. In the two-hour feeding period three of the twenty-one plants were infected after a period of incubation ranging from seventeen to twenty-five days. In the one-hour feeding period none of the twenty-six abacá seedlings developed mosaic.

The results of the three experiments show that *Rhopalosiphum nympheae* was capable of obtaining the virus in two hours of feeding on mosaicked abacá. The aphids which were allowed to feed for one hour only did not cause infection probably because they were still trying to locate a feeding spot and were not actually feeding during the entire period of one hour. According to Stahl and Carsner (1923), the virus of curly leaf of the sugar beet is obtained by *Eutettix tenellus* Baker in ten to twenty minutes. The melon, or cotton aphid, *Aphis gossypii,* obtains the virus (*Marmor cucumeris* Holmes) from mosaicked cucumber in five minutes (Doolittle and Walker, 1928). *Myzus persicae* Sulz., which transmits the cucumber mosaic virus to tobacco, acquires and transmits the virus from diseased to healthy plants in a short time; the entire process is completed in thirty minutes (Hoggan, 1933). The writer found that the abacá mosaic virus (*Marmor cucumeris* Holmes) can be transmitted readily after it is drawn from the host.

Retention of the virus by the aphid vector

Experiment 1. On October 12, 1938, virus-free *Rhopalosiphum nympheae* were allowed to feed on mosaicked abacá for two hours. One hundred of these aphids were immediately transferred to a healthy abacá seedling where they were allowed to remain for one to two hours. From this plant the aphids were transferred to the second seedling where they were allowed to feed for two to three hours. All of the insects from the second abacá seedling were transferred to a third seedling where they were left for twenty-four hours. From the third seedling all the aphids were transferred to a fourth seedling where they were left for ten days. Of these plants only the first seedling showed symptoms of mosaic after an incubation period of sixteen days. The second, the third, and the fourth plants remained healthy throughout.

Experiment 2. On November 11, 1938, the same number of plants and the same number of aphids were used in another experiment. In the second experiment only the first plant in the series showed the mosaic after an incubation period of twenty-one days.

Experiment 3. From December, 1938, to December, 1939, eight other series of tests were made with four healthy abacá seedlings in each test. Of these eight, mosaic appeared only on the plants of three of the tests where the aphids from mosaicked abacá were transferred and allowed to feed first. In one of the tests, mosaic appeared also on the seedling that received the aphids after feeding on the first experimental abacá. In these two abacá seedlings the incubation periods ranged from thirteen to twenty-eight days. In the other four series of tests, none of the plants to which the aphids were transferred from the first seedling to the second became infected with mosaic.

The results of the three experiments indicate that, in feeding on the first plant, all of the virus carried by *Rhopalosiphum nympheae* is left with the plant. These findings corroborate those of Doolittle and Walker (1928) for *Aphis gossypii,* but they differ markedly from those of Stahl and Carsner (1923), who report that *Eutettix tenellus* remains infective throughout its life once it acquires the virus of curly-top (*Chlorogenus eutetticola* Holmes, 1939) of sugar beet. Kunkel (1926) noted that, with age, virus-bearing carriers lose their ability to transmit the disease. Storey (1933) attributes the loss of power of *Cicadulina mbila* to transmit the streak disease of corn

(*Marmor maidis* Holmes, 1939) to an eventual exhaustion of the virus supply in the body of the insect.

In abacá mosaic the acquisition of the virus from a diseased plant and its introduction into a healthy plant was accomplished by *Rhopalosiphum nympheae* in three to four hours. It was noted that the condition of the experimental abacá and the time and temperature of the day influence considerably the transmission of the disease. As a rule young and vigorously growing abacá seedlings with an unfurled leaf at the time of transference of the aphids responded more readily to infection than old and slow growing plants. This was especially true when aphids were transferred early in the morning or during cool and rainy days. When young leaves were available for feeding and the temperature was favorable, the aphids settled down and fed immediately. On warm sunny days, however, the insects moved about considerably.

FAILURE BY THE APHID VECTORS OF ABACÁ MOSAIC TO TRANSMIT THE BUNCHY-TOP OF ABACÁ

Experiment 1. Virus-free individuals of *Rhopalosiphum nympheae* were reared on abacá plants infected with bunchy-top (*Marmor abacá* Holmes, 1939). From March 1 to June 18, 1939, one hundred adults were transferred to each of twenty healthy abacá seedlings. Six plants to which virus-free aphids were transferred served as control. In spite of the large number of aphids employed, however, none of the experimental plants was infected showing that *Rhopalosiphum nympheae* cannot transmit the virus of bunchy-top.

Experiment 2. A similar experiment was performed simultaneously with virus-bearing *Aphis gossypii.* Twenty young abacá seedling were used from March 1 to June 18, 1939, but none of the plants showed bunchy-top infection.

The failure of *Rhopalosiphum nympheae* and *Aphis gossypii* to transmit bunchy-top seems to be due to a definite and specific relationship between the virus of bunchy-top of abacá and *Pentalonia nigronervosa.* The specificity of virus diseases of plants to insect vectors has been shown in many diseases. Smith (1934) considers three groups of insect carriers: (1) mechanical carrier, (2) insect species which have a particular affinity for a plant virus which may be transmitted by other insects, and (3) a specific insect which is capable of transmitting only a definite virus. Kunkel (1926) established the intimate and specific relationship which exists between aster yellows (*Chlorogenus callistephi* Holmes, 1939) and its insect

vector. It is now recognized that even in one-vector species the individuals differ in their ability to transmit the disease (Kunkel, 1926; Storey, 1932). Some viruses (*Galla fijiensis* Holmes) are transmitted by one or only a few closely related species of insects although other insects may have similar anatomical structures which are suited for effective transmission. Storey (1939) pointed out that, in the case of *Peregrinus maidis* and non-infective *Cicadulina mbila* some other mechanism besides impermeability to the virus of the intestinal wall of the insect seems to prevent the translocation of the inoculum into the blood of the vector and, consequently, to inhibit its transmission.

The results of the writer's experiments indicated that the virus of mosaic and the virus of bunchy-top of abacá are transmitted by different insects vectors. That the two diseases are caused by two different viruses is shown by the fact that (1) they exhibit different symptoms, and (2) mosaic infected abacá plants with bunchy-top and the bunchy-top virus readily infected mosaicked plants. This inability of the bunchy-top virus to protect abacá from mosaic is a further evidence that the two diseases are caused by viruses which are not related to each other. This method of determining virus relationship has been established by various workers. Kunkel (1936) found that peach trees infected with little peach (*Chlorogenus persicae* var. *micropersicae* Holmes, 1939) are immune to peach yellows (*Chlorogenus persicae* Holmes), and trees with the yellows are immune to little peach. Since trees infected with one virus are protected from the disease caused by the other, Kunkel (1936) states that these two diseases are caused by strains of the same virus. Similarly, Price (1936) regarded ringspot No. 2 (*Annulus zonatus* Holmes) of tobacco distinct from ringspot No. 1 (*Annulus tobaci* var. *virginiensis*) and eleven other virus diseases of tobacco by means of specific immune reaction. On the same basis Price (1936) believes that the viruses of ringspot No. 1, green-ringspot (*A. tabaci* var. *kentuckiensis* Holmes) and yellow-ringspot (*A. tabaci* var. *auratus*) are closely related.

As the writer considers the Philippine abacá mosaic identical with the banana mosaic in Australia, its causal virus is Musa virus 3 of Magee. This virus is identical with *Cucumis Virus I*, or *Marmor cucumeris* Holmes (1939). The bunchy-top of abacá in the Philippines is caused by Musa virus 2 of Ocfemia (Smith, K. M., 1937), or *Marmor abacá* Holmes (1939).

HOST RANGE OF THE DISEASE

With the use of *Aphis gossypii,* Magee (1940) in Australia has transmitted the virus of mosaic, or infectious chlorosis of banana, from *Musa cavendishii* Lamb. to *Musa ensete* Gmel. and to a seeded species of *Musa.* From the seeded species of *Musa,* Magee (1940) transmitted the virus to the Cavendish and Gros Michel bananas, to abacá, *Canna indica, Cucumis sativus* Linn., *Cucurbita maxima* Duchesne, and *Lycopersicum esculentum* Mill.

Transmission experiments of abacá mosaic to other species of Canna

Experiment 1: *Failure of transmission of abacá mosaic to Canna edulis Ker.* Owing to the prevalence of mosaic of wild *Canna* in Davao, it was suspected that *Canna* might be a possible host of the abacá mosaic. The transmission of the abacá mosaic to *Canna* was determined by the same method used for transmitting the disease from abacá to abacá. Fifty virus-bearing *Rhopalosiphum nympheae* were transferred to each of twenty-five young shoots of *Canna edulis,* grown from seeds, from June 3 to August 12, 1938. On an equal number of healthy *Canna* seedlings, virus-free aphids were reared for control. Although the aphids multiplied on *Canna edulis,* none of the plants became infected with mosaic.

Experiment 2. On September 12 and 19, 1939, ten seedlings of *C. edulis* were used in another transmission experiment with *Rhopalosiphum nympheae* as vector, but none of them showed symptoms of mosaic.

Experiment 3. Transmission of abacá mosaic to Canna indica Linn. Corms of healthy *Canna indica* were secured from Davao through the courtesy of Mr. U. Kojima, and these were planted in pots in the Department of Plant Pathology. As soon as the shoots were fifteen to twenty centimeters tall they were used in transmission experiments with adults of *Rhopalosiphum nympheae.* Ten shoots were used from September 6 to 17, 1938. After ten to twenty days, all the plants on which virus-bearing aphids were colonized developed symptoms of mosaic.

Experiment 4. The experiment was repeated with twelve seedlings of *Canna indica* which were grown from seeds of healthy plants at Los Baños, and 100 per cent infection with mosaic was obtained in nine to seventeen days whereas the control remained healthy.

Experiment 5. Failure of transmission of abacá mosaic to other species of Canna. Fifty virus-bearing *Rhopalosiphum nympheae* were colonized on each of twenty-six plants of an unknown species of *Canna* and on twenty-five of another unknown species. For undetermined reasons the insects moved away from these two species of *Canna* apparently in search of a more suitable plant. The writer did not ascertain whether the insects had fed on the plants or not, but none of the plants became infected.

In some virus diseases infection is not always accompanied by the appearance of visible symptoms. Diseased plants with masked symptoms of virus diseases or plants which are symptomless carriers of viruses are of common occurrence (Elmer, 1925; Johnson, 1925; and Lackey, 1929). The writer determined whether or not *Canna edulis* and the other species of *Canna* which were not infected with mosaic contained virus. The transmission to abacá seedlings of the suspected virus was tested with the uninfected *Canna* plants used as sources of inoculum. As none of the twenty-three abacá seedlings developed mosaic, it was concluded that *Canna edulis* and the other species of *Canna* did not show mosaic symptoms because they did not have the virus.

Failure of transmission of abacá mosaic to different varieties of banana

Experiment 1. Suckers of different varieties of banana were grown in pots. From July 9, 1937, to June 15, 1938, fifty virus-bearing adult *Rhopalosiphum nympheae* were transferred to each of the shoots of sixteen Latundan, *Musa sapientum* var. *cinerea* (Blanco) Teodoro; twenty-one Lacatan, *M. sapientum* var. *lacatan* (Blanco) Teodoro; twenty Buñgulan, *M. sapientum* var. *suaveolens* (Blanco) Teodoro; and twenty-three Saba, *M. sapientum* var. *compressa* (Blanco) Teodoro. In spite of the large numbers of aphids transferred to each banana plant, however, none of the plants developed mosaic.

Experiment 2. With the use of *Aphis gossypii* as vector seventy-five to one-hundred virus-bearing individuals were transferred to each of ten plants of each variety of banana listed above. Although the number of aphids used was increased, none of the plants became diseased.

The mosaic of abacá in the Philippines is doubtless identical with the Australian banana mosaic or infectious chlorosis. In his present study, however, the writer could not transmit the abacá mosaic to the

Philippine bananas. The writer noted that the aphids could not gain a foothold on bananas; hence they could not feed on them.

Failure of transmission of abacá mosaic to camia (Hedychium coronarium K.) and cotton

Repeated parallel transfers of *Rhopalosiphum nympheae* and *Aphis gossypii* from mosaicked abacá failed to infect twenty-five young shoots of camia and twenty cotton seedlings (*Gossypium* sp.).

MECHANICS OF INOCULATION WITH ABACÁ MOSAIC VIRUS BY INSECT VECTORS

The mode of feeding of *Rhopalosiphum nympheae* and *Aphis gossypii* to show how they obtain the virus from diseased abacá and how they inject it into healthy plants was studied in an effort to account for the readiness with which some aphids transmit a disease while others consistently fail.

Preparation of sections for microscopic study

Portions of abacá leaves with aphid colonies on them were cut out and immediately placed in a test tube containing a piece of cotton moistened with chloroform. It was found that the best time to do this was on cool days especially in the morning. At low temperatures the aphids remained quiet and fed actively on abacá leaves. It was possible to cut portions of the leaves with aphid colonies on them and subject the insects to chloroform vapor for a few seconds before they could withdraw their stylets. After the chloroform treatment the leaf sections were fixed in Chamberlains' (1932) formo-aceto-alcohol for four days, washed with water, passed through the usual grades of alcohol, embedded in paraffin in the usual manner and sectioned 15 to 20μ in thickness. Chamberlain's formula was slightly modified by using 90 cc. of 70 per cent alcohol instead of 85 cc., and 5 cc. of commercial formalin instead of 10 cc. The sections were stained overnight with safranin A prepared according to the following formula:

Safranin	1.0	gram
Alcohol, ethyl, 95 per cent	50.0	cc.
Water	50.0	cc.

They were counterstained with gentian violet for a few minutes. The gentian violet was prepared as follows:

Gentian violet	1.0	gram
Alcohol, ethyl, 95 per cent	20.0	cc.
Aniline	3.0	cc.
Water	80.00	cc.

Ziehl's Carbol fuchsin was also tried as a counterstain but it did not give a satisfactory result. This stain was prepared according to the following formula:

Fuchsin	1.0	gram
Carbolic acid	5.0	"
Alcohol, ethyl, 95 per cent	10.0	cc.
Water	100.0	cc.

Good sections of the leaf which showed the insect's stylets were obtained. It was noted that *Rhopalosiphum nymphaeae* and *Aphis gossypii* have a common way of feeding on abacá leaves. Setal penetration of the leaves was accomplished chiefly through the lower epidermis. From the examination of a large number of slides it was noted that the penetration of the leaf by the stylets of the aphids was not always through the stomata. Direct penetration through the epidermis of young leaves was common. The tissues sought for by the stylets appeared to be the phloem of the vascular region (Pl. 4, fig. 1, 2, 3, and 4; Pl. 5, fig. 1, 2, and 3). Sections were obtained in which the setal tips entered the leaves through the lower surface, passed the mesophyll, and almost reached the palisade parenchyma (Pl. 5, fig. 4). In such cases it was noted that the stylets pierced the leaf much more deeply, probably in an effort of the insects to locate the vascular region. Consequently the stylets traversed about two-thirds of the entire thickness of the leaf. There seems to be little difficulty for the sucking mouth parts to pass between the cells. In the majority of the sections examined the stylets reached the phloem and in a few instances the palisade parenchyma by passing between the cells (Pl. 4, fig. 1, 2, 3, and 4,; Pl. 5, fig. 1 and 2) and occasionally through individual cells (Pl. 5, fig. 3 and 4). In cases where the stylets did not pierce the leaf directly toward the phloem, the tips of the setae curved toward the phloem indicating that the insects prefer this tissue (Pl. 4, fig. 1, 2, 3 and 4; Pl. 5, fig. 1 and 3). The penetration of the setae into the phloem corroborates the findings of Büsgen (1891), Brandes (1923), Davidson (1923), Horsfall (1923), Smith (1926), and Tate (1937). Owing to the penetration of the phloem, Tate states that

the main source of food of the aphids is the phloem. Other groups of insects, notably the leafhoppers, feed on the same region (Storey, 1932; Bennett, 1934). Bennett found that the slender stylets of *Eutettix tenellus* Baker passed through the parenchymatous region and reached the phloem with remarkable accuracy. Intercellular penetration to the phloem is typical of the aphids and of Hemiptera with weak mouth parts (Naude, 1926). According to Brandes (1923) the setae of *Aphis maidis* passed through the mesophyll cells of the sugar cane leaf and reached the phloem either intercellularly or intracellularly.

Brandes (1923), Tate (1937), and other investigators report copious salivary secretions from the tips of the insect's stylets at the time of feeding. These authors state that the salivary secretions appear distinctly red when stained with safranin and gentian violet. Tate (1937) was able to demonstrate the stylet sheaths or the path of the stylets owing to the readiness with which the salivary secretions absorb stain. In *Aphis maidis*, Brandes (1923) considers the salivary secretions as the most effective medium through which the virus of sugar cane mosaic (*Marmor sacchari* Holmes, 1939) was transmitted.

In *Rhopalosiphum nymphaeae* and *Aphis gossypii*, the writer noted a thin film around the stylets of the insects. This film hardened and readily stained red after treatment with safranin. In a few instances where the insects had partly withdrawn their stylets, the depth of penetration was clearly indicated by the reddish coloring material that was left behind. The red-staining substance corresponds to what Tate (1937) and others regard as salivary secretions. The presence of small quantities of the readily staining substance was noted in sections where the stylets of the aphids or portions of them were evident. That these reddish coloring sheaths were not a part of the stylets could be seen from the fact that the color of the stylets was clear yellow. It was further noted that in some instances a small amount of the hardened coating of reddish coloring materials peeled off from the stylets without apparent injury to them. The part of the stylets from which the reddish sheaths were removed appeared clear yellow.

The virus of abacá mosaic (*Marmor cucumeris*) is drawn by the aphids during the feeding. The introduction of the virus into healthy plants is accomplished when these virus-laden aphids feed on healthy plants. Although Brandes (1923) believes that the salivary secretion is the medium through which the virus of sugar cane mosaic is introduced into the sugar cane by *Aphis maidis*, Storey (1939) found

that in *Cicadulina mbila* the saliva turns into a gel immediately after ejection so that its suitability as a medium for inoculation is doubtful.

Although the writer noted small quantities of what appears to be salivary secretion from the stylets of *Rhopalosiphum nympheae* and *Aphis gossypii,* its function as a medium for transferring abacá mosaic virus was not studied in this work.

CONTROL MEASURES

Based on laboratory experiments, the following exclusionary and eradicatory measures may be suggested for the control of the mosaic of abacá.

Exclusion

Owing to the destructiveness of mosaic disease and the ease with which it may be transmitted from mosaicked to healthy abacá plants by at least two different species of insect vectors, its spread into an uninfected abacá locality should be carefully guarded against. Exclusionary measures identical with those recommended by Ocfemia (1934) for bunchy-top should be observed. All planting materials such as corms and suckers of abacá should be secured only from fields where the mosaic disease is not present. Leaf sheaths and leaves of abacá, leaves and rootstock of *Canna indica,* and living cotton plants or parts of these from mosaic-infected abacá fields should not be taken into mosaic-free abacá plantations. These materials are dangerous because they may carry infective aphids.

Eradication

Abacá plantations should be inspected regularly at frequent intervals in order to detect the presence of the disease as early as possible. A knowledge of the earliest visible symptoms of abacá mosaic, may save the planter from otherwise laborious and expensive work of eradicating the disease.

When a few plants appear mosaicked or are suspected of being infected with the disease, these plants should be dug up at once and burned in the place of digging (Ocfemia, 1934). In burning the plants there should be produced sufficient heat to render the corms incapable of germination and growth. As a precautionary measure, grasses and other plants and the soil in the immediate vicinity should be sprayed with a liberal amount of soap solution to kill the aphids and prevent them from moving and communicating the disease to plants nearby.

Owing to the fact that the disease is not transmitted to the young by infective adult aphids, the areas from which mosaicked plants have been previously eradicated can be replanted after letting the land rest for a few months. The planting materials, however, must be secured from mosaic-free plantations.

The presence among abacá plants of *Canna indica* and other possible host plants of abacá mosaic is dangerous. Such plants should be removed and destroyed as soon as possible. Plants like cotton, tomato, melon, and other probable hosts may not only be dangerous sources of inoculum of abacá mosaic or of a related virus disease but are also natural hosts of *Aphis gossypii*. If these plants are present in the field they should be carefully watched and should not be allowed to grow in the plantation where abacá mosaic is present.

SUMMARY

1. Mosaic is a destructive disease of abacá (*Musa textilis* Née). It is caused by *Cucumis Virus I*, or *Marmor cucumeris* Holmes. The disease is easily communicated to healthy plants by *Rhopalosiphum nympheae* (Linné) and *Aphis gossypii* Glover.

2. The aphid vectors of abacá mosaic cannot transmit the virus of bunchy-top of abacá (*Marmor abacá* Holmes) probably because of a definite and specific relationship between this virus and *Pentalonia nigronervosa* Coq.

3. The abacá mosaic virus cannot be transferred to healthy abacá plants by *Pentalonia nigronervosa* Coq., *Ferrisia virgata* (Cockerell), and *Stephanitis typica* (Distant). Neither can it be transmitted by needle-pricks and by sap injection.

4. The earliest symptom of mosaic appears eight to ten days after virus-bearing aphids have fed on healthy plants. The first visible indication of infection consists of small and yellowish white dots on the unfurled or newly expanded leaf. These circular yellowish white areas elongate into short narrow dashes but their central dot-like beginnings remain distinct. Typical mottling is shown later in the leaves that follow. In advanced stages of infection mottling appears also on the petioles and on the pseudostems of succeeding leaves.

5. Eight adult *Rhopalosiphum nympheae* are sufficient to inject into a young abacá seedling an infective dose of abacá mosaic.

6. The abacá mosaic virus in the adult *Rhopalosiphum nympheae* is not transmitted to the young. It takes the aphid vectors two hours of feeding on mosaicked abacá in order to obtain the virus. The aphid

carriers of abacá mosaic do not retain the virus for a long time. They lose all of the virus in the first feeding on a healthy abacá.

7. Abacá mosaic infects *Canna indica* but not *C. edulis,* and other species of *Canna,* cotton, and *Hedychium coronarium* K. Owing perhaps to the failure of the vector aphids to gain a foothold on the petioles and leaves of Philippine bananas, the writer could not secure transmission of the abacá mosaic to bananas.

8. *Rhopalosiphum nympheae* and *Aphis gossypii* penetrate young abacá leaves either through the stomata or directly through the lower epidermis. The insects' stylets pass between the cells and occasionally through the cells in order to reach the phloem.

9. The exclusionary and eradicatory measures recommended for the control of bunchy-top of abacá are applicable to abacá mosaic.

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DESCRIPTION OF PLATES

PLATE 1

The earliest symptoms of mosaic infection obtained in transmission experiment. Note the small and pale white dots and short narrow dashes on the leaf blade. Nearly natural size. Photographed by the Photographic Division, College of Agriculture, on September 3, 1938.

PLATE 2

The advanced symptoms of mosaic infection obtained in transmission experiment. Note the characteristic mottling on the leaf blade. Nearly natural size. Photographed by the Photographic Division, College of Agriculture, on September 12, 1938.

PLATE 3

Fig. 1. The light brown aphid *Rhopalosiphum nympheae* (Linné) on a one year old abacá seedling. Nearly natural size. Photographed by the Photographic Division, College of Agriculture, on July 6, 1938.

Fig. 2. Individuals of *Aphis gossypii* Glover on leaves of a young abacá seedling. Nearly natural size. Photographed by the Photographic Division, College of Agriculture, on May 25, 1938.

PLATE 4

Photomicrographs of cross-section of abacá leaves with the stylets of *Rhopalosiphum nympheae* (Linné).

Fig. 1. The stylet pierced the leaf in the direction of the vascular bundles. The tip bent in the direction of the phloem as may be seen in fig. 2. About × 400.

Fig. 3 and 4. The stylets between the cells of the phloem. The characteristic stylet sheath may be seen in fig. 3. About × 400.

PLATE 5

Photomicrographs of cross-section of abacá leaves with the stylets of *Aphis gossypii* Glover.

Fig. 1, 2, and 3. The stylets in the phloem region. In fig. 1 the stylet may be seen between the cells; it appears branched on account of the sheaths which formed in the different places explored by the stylet. In fig. 2 the lower epidermis of the leaf sagged down probably on account of the pressure exerted by the penetrating stylet. About × 400.

Fig. 4. The stylet passed through the mesophyll region and tapped the cell in the immediate vicinity of the vascular region. About × 400.

PLATE 1

PLATE 2

PLATE 3

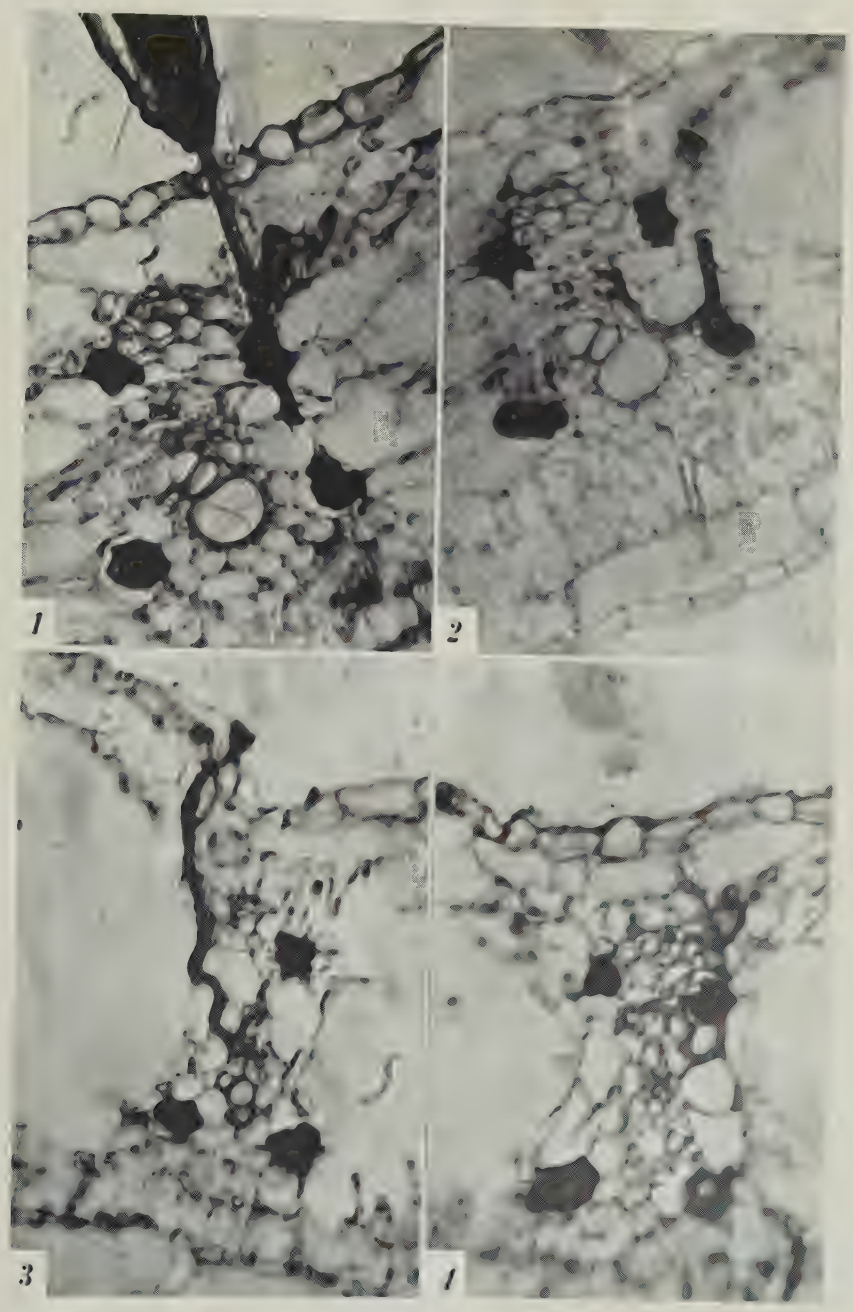

PLATE 4

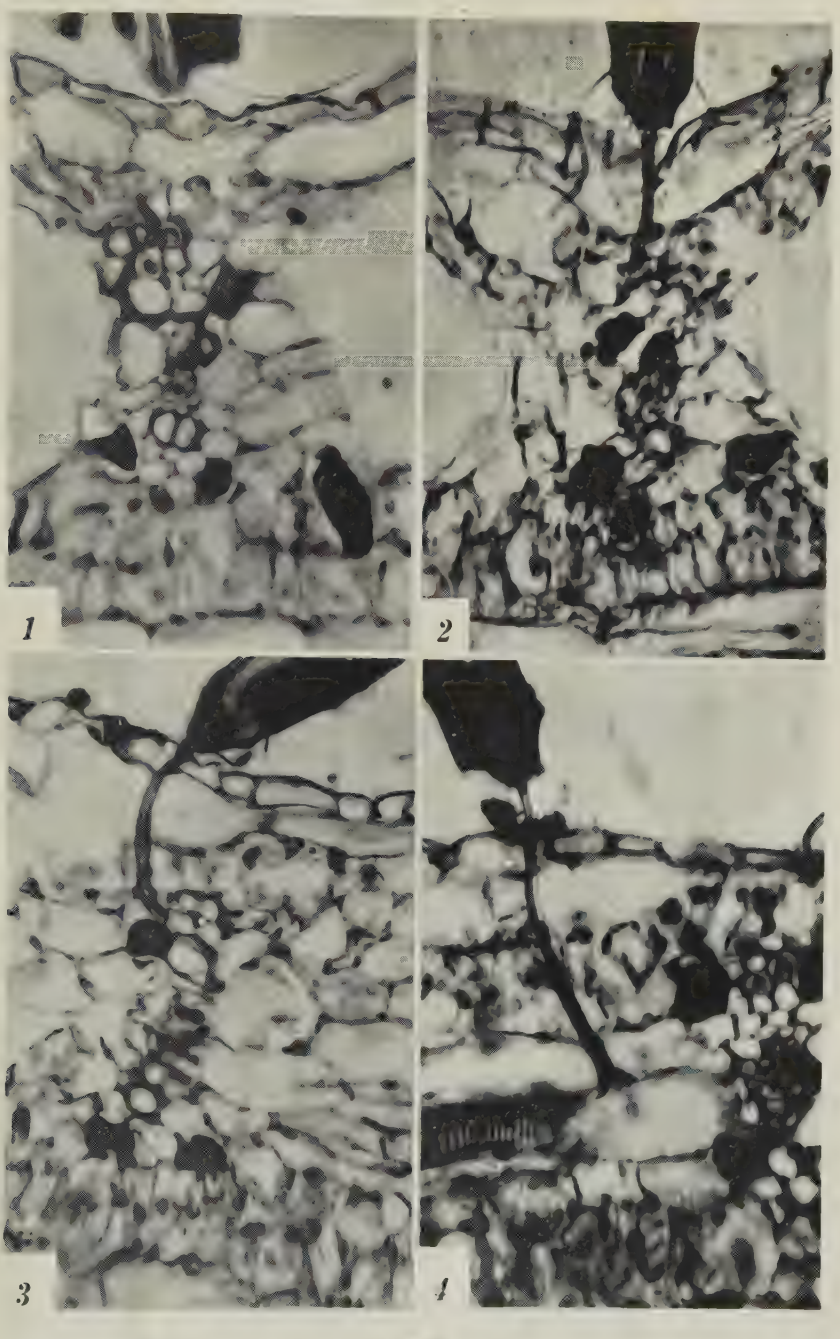

PLATE 5

INTRODUCTION AND TRIAL CULTURE OF AMBON BANANAS IN THE COLLEGE OF AGRICULTURE[1]

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WITH TWO TEXT FIGURES

Previous to 1927 when the present work was begun, Ambon banana or its relatives were introduced in the College three times—the first time on December 21, 1910, involving Bluefields banana, from Lamao Station of the former Bureau of Agriculture; the second, on February 4, 1913, also Bluefields from Cuba; and the third, on September 3, 1915, again from the Bureau mentioned, involving Ambon from Java. The first importation was given the College accession number 123, the second 1371, and the third, 5136 and 5224. Accession number 5136 covered 4 suckers, and No. 5224, 3 suckers. The number of plants covered by the others was not recorded.

It is probable that plants of all these introductions failed to survive, since Teodoro (1915) and Quisumbing (1919), who made studies of bananas found in the Philippines, made no mention of this variety in their reports either under the name Ambon or under any of its aliases. And Harland (1928), after touring the East in 1925 and studying the variety situation in the Philippines in consultation with our former Professor Otto A. Reinking, who had stayed here for some time for the same purpose, claimed that Gros Michel, considered identical with Ambon, did not exist in this country. Furthermore, the Director of the Bureau of Plant Industry of the Philippines, in his answer dated August 7, 1939, to the senior author's inquiry, stated that his Bureau was not aware of the existence of Ambon banana in the Philippines except in the College of Agriculture and in Los Baños Economic Garden, which was only started in October, 1931.

Objects of the work

The objects of this work were to re-introduce the Ambon banana into the Philippines and to carry out a trial culture of it on the

[1] Paper read in the Los Baños Biological Club, July 25, 1940. Experiment Station contribution No. 1379.

College farm. It was also the object of this study to determine the possibility of crossing this banana with our Buñgulan and Latundan. It was considered necessary to include this work in our breeding program as there was then considerable interest in banana circles in the production of a banana hybrid that is resistant to the banana wilt and has the good qualities of the Gros Michel variety, and another hybrid that would have the local cultural adaptability of the Latundan but would at the same time be suitable for export.

Time and place of the work

This work was begun in 1927. Hence, it has been going on for more than thirteen years. It started in Buitenzorg, Java, and was continued at the College of Agriculture, Los Baños, Philippines.

AMBON BANANAS IMPORTED

There are in Java several bananas which bear the name Ambon. The most important of these for export are Ambon Poetih, Ambon Idjo, and Ambon Loemoet. The last one is the least adapted for export as it requires refrigeration in long transit whereas the other two do not. There are also the less common Ambon Bodas and Ambon Garaita. Java has also another export variety known there as Songgroito and Sri, grown specially in Palembang district. The materials of this study consisted of Ambon Poetih, Ambon Idjo, and Ambon Loemoet. Table 1 gives the characteristics of the bunches of these three varieties. In banana literature and in the present report, unless otherwise specified, the Ambon banana meant is Ambon Poetih.

According to Ochse (1925) and (1926), Ambon Poetih and Ambon Idjo are mostly grown in Banjoewangi for export to Australia. Suckers about a man's height are used for planting. They are distanced 4 by 5 meters allowing intercropping with rice. The plants produce one bunch each in the second year. From this time until the sixth year, it is possible to harvest two bunches per stool. After the sixth year, the planting is renewed by digging the old stools and making a new planting between the old rows. This new planting is sometimes done one year before the removal of old stools in order not to have a year without production. Yield is controlled by thinning the suckers in a hill.

While Ambon bananas are sold in fruit markets of Java for local consumption, large quantities of them are exported to West Australia. Ochse and De Jong (1931) gave the following figures on banana exports for the period 1913 to 1930 inclusive. For 1913 to 1915, 170,000 bunches; 1916 to 1920, 506,000 bunches; 1921-1925, 675,900 bunches; 1926-1930, 777,465 bunches.

Besides being grown for consumption as fresh fruit, Ambon Poetih has also been used in Java for fig production, being one of the favorite varieties for this purpose though not so well liked as the Mas banana.

IDENTITY AND RELATIVES OF AMBON BANANAS

For convenience we may place the countries in which Ambon bananas and their relatives are found in two groups. The first is the West Indies and Caribbean group and the second the Indo-malayan group. The Ambon banana, which is the Ambon Poetih, is considered very closely related to, if not identical with, the Gros Michel of the West Indies and Caribbean regions. This variety is also known under different names in different countries such as Bluefields, Fiji, and Jamaica. Among those who believe in this identity are Sands (1925), Harland (1928), and Cheesman (1932).

In Indo-malaya, Ambon Poetih is not known as Gros Michel. It is known as Embon or Embun in Malaya, Klui Hom in Siam, Anamalu in Ceylon, Thihmhwe in Burma; that is, some believe these varieties are the same. Certainly they are closely related; at least they have important fruit qualities in common. Considering that the origin of the banana is Southeastern Asia and that Ambon Poetih is found in the Indo-malayan countries named, it is strange that it is not found as an old variety in the Philippines, although we have what is undoubtedly one of its relatives—the Buñgulan. The relatives of Buñgulan are the Ambon Idjo and Ambon Loemoet of Java, Embun Hijau of Malaya, Klui Hom Kieu and Klui Hom Chan of Siam, and certain varieties in Burma. According to Cheesman and his coauthors (1933), a variety received by them indirectly from the Philippines and labeled Lacatan is identical with Ambon Loemoet. This is evidently a case of mislabeling, for, as we know, Lacatan matures yellow while Ambon Loemoet matures green.

To prevent nomenclatorial confusion inasmuch as we are not sure that Ambon Poetih is identical with Gros Michel or the varieties found in Indo-malayan countries and supposed to be identical with it, it is advisable in introduction work with Ambon bananas and their relatives to use in the importing country the names of different varieties under which they are known in their countries of origin.

NEW INTRODUCTIONS OF AMBON BANANAS

Ambon Poetih. Two corms of this variety were collected by the senior author in Buitenzorg in March, 1927, and mailed to the Philippines to Dr. José M. Capinpin, then in charge of the Plant Breeding Division. Before the corms were mailed, they were coated thickly with powdered charcoal and wrapped in strong paper. The corms were received here on the following April 7. They were given accession number C. N. 18604 and planted immediately under nursery conditions.

On April 2, 1932, six suckers of the same variety arrived in Manila consigned to this College from Pasar Mingoe Station of the Dutch East Indies Department of Agriculture, Commerce, and Industry. These suckers were kept in the quarantine station of the Bureau of Plant Industry at Port Area in Manila. They were released from quarantine on December 31 of the same year but were not brought to the College until January 24, 1933. The plants were given accession number C. N. 19196 and planted in the Plant Breeding Garden two days later.

Ambon Idjo. Five suckers of this variety were received at this College. They belonged to the same shipment as introduction C. N. 19196. The Ambon Idjo suckers were accessioned as C. N. 19195 and planted in the Plant Breeding Garden on January 26 also.

Ambon Loemoet. Five suckers came to the College at the same time and from the same source as C. N. 19195 and 19196. They were given number C. N. 19198 and also planted in the Garden of the Plant Breeding Division on January 26, 1933.

Culture. The trial culture of Ambon Poetih, C. N. 18604, was carried on first in a lightly shaded portion of the Plant Breeding Garden where the soil is clay loam and fairly well-drained. At this place, the suckers which grew from the introduced corms were planted. As soon as suckers had grown to a height of about a meter and when the time was favorable, they were transplanted in the same

lot. This operation was repeated as often as suckers became available. The plants were not fertilized, nor pruned.

On June 15, 1932, the entire culture was transferred to a place on the bank of Boot Creek where it was more shaded and more protected from the wind. This new place was previously planted to Latundan banana which had disappeared owing apparently to the attack of the wilt disease. By planting the Ambon banana here we were able to carry on a field test of the resistance of this variety to this disease. Here also were cultured the other Ambon Poetih plants as well as Ambon Idjo and Ambon Loemoet which were introduced later.

TRIAL CROSSES AND SELF-POLLINATION

In this study only Ambon Poetih was used as female and Buñgulan and Latundan varieties as male parents. The crossing was done on March 24 and 25, 1928, with the help of Mr. Juan O. Unite, then a graduate assistant in Agronomy. Sixty-three female flowers of Ambon Poetih were pollinated with Buñgulan, while 94 flowers were pollinated with Latundan. Thirty flowers were used for self-pollination work. The following is a brief description of the manner in which cross- and self-pollination work was performed.

A day before the flowers of the first hand came out, one entire inflorescence or bunch of Ambon was enclosed in a cloth bag made for the purpose. The bag was made of cheese cloth and was about 40 cm. in diameter and about 120 cm. long. Both ends of the bag were left open. The bunch for pollination work was placed in the bag and then the upper skirt of it was tied to the base of the inflorescence with a piece of string, thus closing the upper end.

In pollination the lower end of the bag was rolled upward until the flowers to be pollinated were exposed. Pollen grains were collected from the male inflorescence of the male-to-be variety and were applied carefully on the stigma of the female flowers with the aid of a pointed stick or needle. When the pollination work was over, the lower skirt of the bag was tied with a string. The inflorescence was kept in the bag for several days after which the bag was removed and the fruit allowed to mature.

In self-pollination, the same technique as that described was used except that instead of using another variety as the source of pollen, a plant of the same variety, the Ambon Poetih, was used. Ambon Poetih being a clon, crossing between its plants is, as a rule, equivalent

to self-pollination of a plant. Self-pollination of a plant in banana is generally difficult to accomplish because, within a bunch, the female flowers mature much earlier than the male.

RESULTS AND DISCUSSION

The results given below are for Ambon Poetih and Ambon Idjo only. Ambon Loemoet died early in the trial. It will be recalled that, originally, five suckers of this variety were planted in the Plant Breeding Garden on January 26, 1933. Of these five suckers, four died. The remaining sucker grew until it formed a clump containing seven stalks. Two of these stalks produced bunches but these never reached maturity. The hill was blown down by the typhoon which occurred in November, 1936, and it was divided into seven planting units and transplanted in the same lot shortly after the typhoon. Of these seven, only two plants lived, and only for some time. One of them died within less than one year after transplanting and the other died after about a year. At present, therefore, we do not have a single plant of Ambon Loemoet in our culture.

Height of plant and diameter of stalk in fruit

When grown in a heavily shaded place, the mature plant of Ambon Poetih reached an average height of 3.7 meters, measuring from the ground to the curvature of the fruit stem, while in a lightly shaded location it averaged 3.0 meters (table 2). These heights are quite below those reported for Embon in Malaya, 4.57 to 6.1 meters (Sands 1925), and for Gros Michel in Central America, 5.48 to 7.62 meters, and in Cuba, 3.66 to 4.57 meters (Reynolds, 1931). However, it is quite possible that these heights include the height of the crown. Plants of Ambon Idjo appear to be taller and thicker than the Poetih. Several stalks measured averaged 4.6 meters in height up to the curvature of the fruit stalk, or 5.8 meters if measured to the top of the crown.

The average diameter of the fruiting stalk of Ambon Poetih in the half open field was 19.7 cm. and in the shade, 21.5 cm. In the half open situation, the stalk could usually support the weight of the bunch it produced; but when grown in the shade, the bunch was heavier and the stalks sometimes broke. It was necessary to provide it with a support to prevent breaking until the fruit matured.

The authors observed further that the entire plant of the Ambon Poetih has a tendency to fall down easily instead of breaking as do

other varieties, and the breaking of the stalk appeared less frequent than in the case of Buñgulan. Ambon Idjo stalks averaged 23.5 cm. in diameter.

Weight of bunch and number of hands and fingers in a bunch

The first two original corms of Ambon Poetih produced large bunches. Planted on April 7, 1927, one of the plants flowered on January 16, 1928, and the bunch of fruit produced was harvested on May 4, 1928. It weighed 23.1 kgm. and consisted of 7 hands and 102 fingers (table 3). The fruit of the other plant was harvested on July 2, 1928. It weighed 24.1 kgm. and contained 9 hands and 157 fingers (fig. 1). The bunches produced subsequently were usually not so heavy. As table 3 shows, the average weight of the marketable bunch was 8.6 kgm.

The bunches produced from 4 to 9 hands each giving an average of 5.9 hands to a bunch. The number of fingers contained in each bunch varied from 42 to 159, averaging 71.2.

To obtain an idea of how the Ambon bunch produced locally compares with those of our common banana varieties, bunches of these produced from time to time were weighed and the hands and fingers counted (table 4).

Table 4 shows that Ambon Poetih produced bunches almost as heavy as Saba, the ranking variety in this character, but not so many hands and fingers. Ambon Poetih in fact ranked fourth in these respects although the differences in some cases do not seem to be very important. Ambon Idjo produced the heaviest bunches but not the largest number of hands and fingers (fig. 2).

Self- and cross-compatibility with Latundan and Buñgulan

In the 30 flowers of Ambon Poetih, self-pollinated, not a single seed was produced; neither was any seed obtained from the crosses made with Latundan and Buñgulan varieties. Cheesman (1931) reported that when two bunches of a variety labeled, Bumulan, which came from the Philippines, were pollinated with Gros Michel, not a single seed was produced. The same result was obtained when one bunch of a variety labeled Lacatan was pollinated with Gros Michel. Ambon Poetih, as well as Ambon Idjo, is most likely cross-incompatible with most banana varieties since here, as well as in Java, these varieties produced seedless fruit in spite of the fact that they were grown where many other varieties were in culture.

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If we are to continue in our attempt to produce an Ambon type of banana that is resistant to the serious diseases affecting Gros Mi-

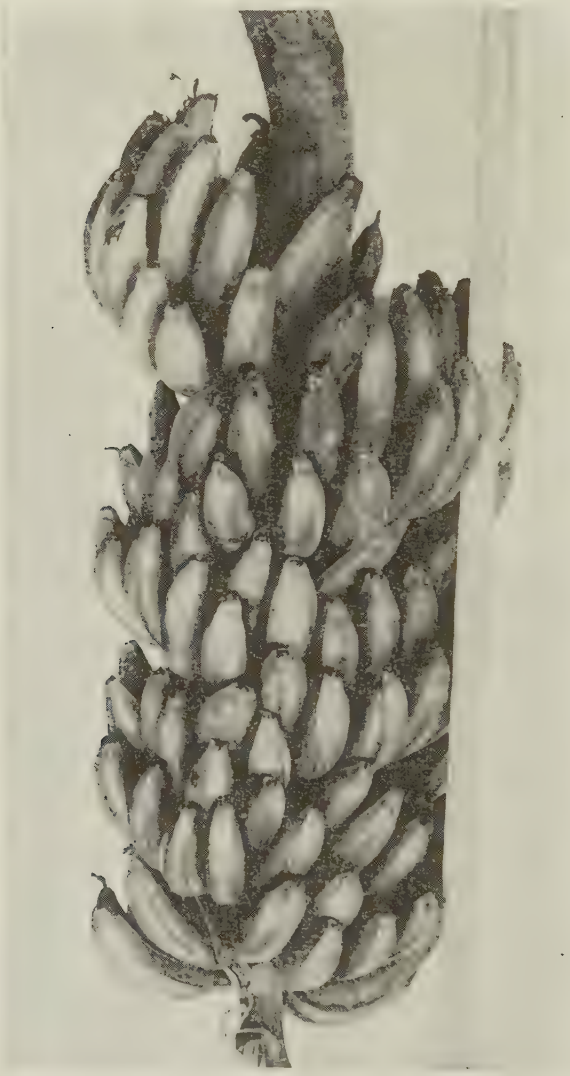

Fig. 1.—A bunch of Ambon Poetih fruits produced on the College of Agriculture Farm. This bunch weighed 24.1 kgm. and had nine hands and 157 fingers. Photograph by the Photographic Division, College of Agriculture.

chel and a Latundan type that, through hybridization, would be more adapted for exportation, it would be necessary to introduce more of

the Ambon bananas from the East as it is not impossible that some of these may be cross-fertile with our Latundan and Buñgulan. Even

Fig. 2.—A bunch of Ambon Idjo fruits produced on the College of Agriculture Farm, weighing 21.7 kgm. and containing nine hands and 143 fingers. Photograph by the Photographic Division, College of Agriculture.

if all of them should prove similarly cross-incompatible, there is the remaining possibility of one of them proving a resistant kind. Our

own results with Ambon culture tend to strengthen this possibility as will be pointed out later.

Market and food values

In the Department of Agronomy bananas are sold by the hundred, Ambon bananas being priced as high as Lacatan. Both are sold at ₱0.40 per hundred of good sized fruit while the other varieties, at lower prices—Buñgulan at ₱0.35, Latundan and Gloria at ₱0.30, and Saba at ₱0.25. In the Coconut Grove, we once saw a bunch of Ambon Poetih being offered for sale at the same price as Buñgulan. What the price was on the basis of 100 bananas we could not learn.

Using the prices given above and the data on number of fingers per bunch of the common varieties given in table 8, we find the market value per bunch of the different varieties to be as follows: Ambon Poetih, ₱0.285; Ambon Idjo, ₱0.356; Lacatan, ₱0.360; Buñgulan, ₱0.353; Latundan, ₱0.168; and Saba, ₱0.315.

Until we know the comparative production per unit area of land of these different varieties, it would be hard to venture a guess as to which variety would be most profitable to grow. Assuming equal productivity, the last table shows that Ambon Poetih would bring less income than the other varieties except Latundan. Ambon Idjo would give about the same income as the other varieties and better income than Latundan.

It is quite probable that the Ambon bananas could be sold at much higher comparative prices in Manila groceries which are patronized by the large hotels and resident foreigners, as there is undoubtedly a decided preference for these bananas among the Americans and possibly the Europeans. In 1931 we gave suckers of Ambon Poetih to the Post Garden of Fort William McKinley; subsequently the Post Garden officer wrote to us and stated that the flavor was very superior to others. He expressed a desire to buy 500 more suckers.

Once we sent a bunch of Ambon Poetih to Manila for pricing by different grocery stores. Our agent, however, was told that unless it could be supplied regularly in desired quantities, it was impossible to quote price.

The food value of Ambon Poetih fruit produced on the College Farm was studied by a senior student, Feliciano Nuestro,[2] under the

[2] Nuestro, Feliciano. Proximate analysis of twenty-nine varieties of banana grown in the Philippines. Thesis presented for graduation in the College of Agriculture. 1931. (Unpublished).

direction of Professor Francisco O. Santos. Nuestro determined the proximate chemical composition of 29 varieties of bananas grown in the Philippines. From the results of his analyses, we are quoting in table 5 those pertaining to Ambon Poetih and the important bananas of the country.

Table 5 shows that Ambon Poetih was found to have a lower calorific value than the bananas commonly consumed in Philippine homes. This disadvantage, however, is partly offset by its higher percentage of edible portion and its superior eating quality.

In connection with the food value of Ambon Poetih, it should probably be stated here that in Java it has a certain use which we do not find in other bananas. According to H. J. Wigman, Sr.[3] this banana is recommended and employed as the foremost regular feed for children who can not secure enough milk from their mothers. He considered this use of Ambon banana as arising from its low content of fiber and starch. He said that the delicate intestines of the children not only tolerate this food but are also mildly stimulated by it.

Ochse (1924) cites Dr. Boorsma's analyses of 17 varieties of Java bananas as giving 0.19 per cent fiber for Ambon as against 0.22 to 0.87 per cent for 15 others. The only variety that had lower fiber content than Ambon was Radja Sijam which had 0.18 per cent. Nuestro found the percentage of fiber in Ambon Poetih fruit to be 0.23 as against 0.38 to 0.67 per cent of the others named in table 9.

Resistance to the wilt disease and to pests

Under the climate and soil conditions of the College where this plant has been grown for over twelve years, Ambon Poetih has not been observed infected with the banana wilt although the place was once occupied by Latundan plants which suffered the attack of this disease. Also attacks by serious pests have not been noticed. The same may be said of Ambon Idjo.

Size of present cultures

At present we have in our cultures 325 hills of Ambon Poetih and 20 hills of Ambon Idjo. From these hills about 205 suckers of Ambon Poetih and 17 of Ambon Idjo are available for transplanting. The authors do not know the size of the present cultures of those who have obtained planting materials of Ambon Poetih from the College.

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[3] WIGMAN, H. J., Sr. (year and publishers not given). Ooftteelt, pp. 583-658.

In June, 1933, the Post Garden officer of Fort William McKinley wrote to the College that the five suckers given them in August, 1931, had produced 10 new suckers each, or a total of 50 suckers. At this rate of propagation, there ought to be a good sized planting of banana in Fort William McKinley if the Post made it a point to enlarge its culture.

Suckers of Ambon Poetih have been distributed to planters in Laguna, Batangas, Rizal, and Iloilo. No sucker of Ambon Idjo has been distributed so far.

SUMMARY

The College of Agriculture imported Ambon bananas from Java in April, 1927, and in April, 1932. These bananas are Ambon Poetih, Ambon Idjo, and Ambon Loemoet. The last variety did not survive the trial and no longer exists on the College Farm. Ambon Poetih is now represented in College cultures by 325 hills, and Ambon Idjo, by 20 hills.

Under our local soil and climate conditions, Ambon Poetih plants have attained an average height of 3 to 3.7 meters, measured from the ground to the curvature of the fruit stem, a trunk diameter of about 19.7 to 21.5 centimeters and have produced fruit bunches, weighing on the average 8.61 kilograms and containing about 6 hands and 71 fingers. Ambon Idjo plants have attained an average height of 4.6 meters and trunk diameter of 23.5 centimeters and have produced bunches weighing 11.97 kilograms and containing about 7 hands and 89 fingers on the average. Ripe Ambon fruits produced locally are seedless, a characteristic of these bananas, have fine flavor, and are well liked by those who have tasted them, particularly the Americans, the latter expressing preference for these bananas to the other varieties usually sold in the market. The proximate chemical composition of Ambon Poetih fruits has been determined by the Department of Agricultural Chemistry.

Ambon Poetih and Ambon Idjo have not shown any disease symptom similar to those produced by the banana wilt. They have been grown for at least ten years in a place where previously Latundan banana had been grown and badly attacked by this disease.

Ambon Poetih has been found naturally as well as artificially self-incompatible and also cross-incompatible with our Buñgulan and Latundan bananas.

RECOMMENDATIONS

From the results of this study, the authors conclude that it is now safe to recommend a wider trial of Ambon Poetih in the Philippines. For these results to be of more lasting benefit they would recommend that about 15 suckers of this banana be sent to each important banana province for trial and subsequent distribution under a cooperative and experimental basis. There are eight of these provinces, namely, Batangas, Bohol, Cebu, Iloilo, Occidental Negros, Oriental Negros, Pangasinan, and Zamboanga. We have about 200 suckers available for distribution, and if we give 15 suckers to each of these provinces, we would still have about 80 left for other uses.

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TABLE 1

Characteristics of bunches of three different Ambon bananas [a]

PART STUDIED	VARIETY		
	Ambon Poetih	Ambon Idjo	Ambon Loemoet
Stem of fruit	Thickly haired and blackish at upper point of fruit stalk, elongated.	Entire fruit stem and upper point shorter than those of Poetih	Under part of stem small, short, and slightly haired; blackish, short fruit stem
Spacing between hands	large	large	wide
Fingers	large	smaller than Poetih	small, long and round
Ripe fruit	white yellow	light greenish yellow	green, sometimes yellowish green
Skin of fruit	thick, separates easily when ripe	similar to Poetih	thick, black, speckled
Flesh	yellowish, sweet with little aroma	similar to Poetih	rose colored
Top end of fruit	large and flat	ends of fingers less flat and sometimes pointed	large and flat
Position of fruit	regularly arranged	not as regularly arranged as Poetih	

[a] Compiled and translated from Ochse's (1926) article.

TABLE 2

Height and diameter of fruiting stalk of Ambon Poetih

CHARACTERS STUDIED	AVERAGE
Height of fruiting stalk in meters:	
Lightly shaded [a]	3.02 ± 0.16
Heavily shaded [b]	3.70 ± 0.29
Difference	0.68 ± 0.32
Greatest diameter of stalk in centimeters:	
Lightly shaded [a]	19.76 ± 0.20
Heavily shaded [b]	21.52 ± 0.28
Difference	1.76 ± 0.34

[a] Based on measurements of 15 typical stalks which matured in the same week.

[b] Based on measurements of 12 typical stalks which matured in the same week.

TABLE 3.

Some fruit characters of Ambon banana

WEIGHT OF BUNCH			NUMBER OF HANDS PER BUNCH			NUMBER OF FINGERS PER BUNCH		
Class value	Frequency		Class value	Frequency		Class value	Frequency	
	Poetih	Idjo		Poetih	Idjo		Poetih	Idjo
kgm.			*hands*			*fingers*		
5	22	1	4	16	0	42	14	1
7	29	5	5	25	4	55	22	3
9	22	1	6	38	6	68	22	2
11	15	3	7	28	6	81	62	6
13	3	2	8	2	2	94	20	2
15	5	3	9	2	3	107	7	3
17	2	4				120	3	2
19	2	0				133	0	1
23	1	1				146	1	1
25	2					159	1	
Total	103	20		121	21		112	21
Average	8.61	11.97		5.9	6.6		71.2	88.9

TABLE 4

Average number of hands and fingers and weight per bunch of some important varieties of banana grown in and near the College

VARIETY NAME	AVERAGE WEIGHT PER BUNCH	HANDS PER BUNCH	FINGERS PER BUNCH
	kilogram	*number*	*number*
Ambon Poetih ..	8.6	5.9	71.2
Ambon Idjo ...	11.5	6.6	88.9
Latundan	4.3	5.3	56.2
Buñgulan	8.2	8.2	101.0
Lacatan	6.5	6.5	90.9
Saba	9.2	7.8	126.0

TABLE 5

Proximate composition of Ambon compared with important Philippine bananas

VARIETY	EDIBLE PORTION	COMPOSITION OF EDIBLE PORTION							
		Moisture	Crude fiber	Protein	Crude fats	Starch	Water sol. red. sugar	Total carbohydrate	Calories per 100 grams
Latundan	71.7	69.94	0.48	1.13	0.53	8.99	9.35	26.35	118
Lacatan	62.4	70.76	0.38	1.19	0.69	8.32	12.75	25.78	117
Buñgulan	64.3	69.21	0.50	1.98	0.72	6.53	15.57	26.75	124
Saba	53.4	70.72	0.67	0.75	0.50	16.74	8.51	26.11	115
Ternate	67.5	69.44	0.59	0.97	0.73	5.33	15.31	27.35	123
Morado	73.1	71.37	0.61	1.11	0.56	8.99	10.46	25.40	114
Ambon Poetih .	68.9	73.63	0.23	1.02	0.44	3.24	11.75	23.49	105

A STUDY OF THE GROWTH HABIT OF RICE PLANTS: V. FERTILIZATION AND GRAIN SIZE [1]

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AND

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WITH FOUR TEXT FIGURES

This paper represents the last of the series of articles (Apaoan, 1939; Juliano, 1938, 1939, 1940) on the growth habit of rice plants. It deals especially with the effect of fertilization on the weights of unhulled and hulled grains, dimensions of the hulled grains, tillering habit, booting and flowering durations, average height of the plants at maturity, length of panicles, and number of filled and unfilled grains per panicle when grown in tin cans or in the field. The paper also presents evidences to prove that size of hulled rice grains is the most stable of all characters of the rice plant.

MATERIALS AND METHODS

Preparation of the land and tin cans. The land utilized by Juliano (1938) and located on the eastern side of the Department of Agricultural Botany, College of Agriculture, was dug with a spading fork and mattock on December 13, 1937. The ground was divided into two equal lots with about half a meter of ground left untilled around and between them to serve as dikes. The lot towards the north was higher than that towards the south. After both lots were prepared, water was run into them, and the soil was puddled and levelled with the feet and garden hoe.

The dike which ran from east to west and separated the two lots was reinforced on its two sides with a plain galvanized iron sheet

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which was driven down until it reached the hard pan in order to minimize seepage of the irrigation water from one lot to the other. At one end of this dike a cut was made so as to allow five inches of irrigation water to remain in the north plot. Another cut for drainage at the other end was made as far down as the surface of the ground.

Fifty-eight tin cans were cleaned, dried, and their bottoms painted with dehydrated coal tar on the outside. When the paint had dried, the cans were filled with soil obtained from the prepared lots to about 10 centimeters from the top. Two holes about three millimeters in diameter were made on one side of each can, one five centimeters from the top, and the other at the level of the surface of the soil. These holes were for maintaining constant the level of the irrigation water in the cans and for drainage. The cans were placed in four rows on the south side of the Agricultural Botany building, about ten meters from the field plots, and fenced with a wire netting. A similar enclosure of wire netting was provided for the field plots.

Seeding and transplanting. Seeds of the pure line Guinangang rice variety obtained from the harvest made by Juliano (1938) were planted in two of the prepared tin cans on December 9, 1937. Previous to sowing, the seeds were soaked in water over night. On December 20, 1937, eleven days after seeding, the seedlings were transplanted.

Four seedlings were planted in each can, one to each corner. Each can and plant was labelled carefully. In the cans 232 plants were set out.

In the field the seedlings in each of the two lots were transplanted in fifteen rows of twelve hills each; the hills were spaced similarly to those planted in cans. In each of the two lots 180 plants were set out. The individual rows and the hills in each row were labeled separately.

Application of fertilizer. The fertilizer used in this study was ammonium sulfate containing 20.6 per cent of nitrogen. The rate of application was 4.71 grams for every four plants as found good by Almeda (1937) for an upland rice (Inintiw). The application was made only once, on January 1, 1938, eleven days after transplanting and when the seedlings were twenty-two days old. In the can culture, only half were fertilized; the other half served as control. In the field culture, the lot which was lower and located on

the south side was the one fertilized, while the one on the north side served as control.

Irrigation. To irrigate the plants, water was taken from a faucet with a rubber hose. Plants grown in cans were watered every afternoon. Water was supplied continuously to the field plants in the northern lot which was six inches higher than the fertilized one, and the water that overflowed served to irrigate the latter lot. The irrigation water was kept at a constant depth of five centimeters both in the can and field cultures throughout most of the growing period of the plants. Whenever necessary, it was withdrawn simultaneously from the can and field cultures, and when the plants were nearing maturity it was withdrawn completely.

Weeding. Weeding was done by hand whenever any weeds appeared in any of the cultures.

Tillering. Weekly counts were made of the number of visible tillers per hill or plant in cans and in the field. This observation was begun on January 2, 1938, but inasmuch as only a few of the hills in either culture actually showed any tillering, actual counting was only started on January 9, 1938, a month after sowing and twenty days after transplanting. Eight weekly counts were made for both cultures.

Booting and flowering. Records of the time of booting and inflorescence emergence of the individual culms in each hill were kept.

Harvesting. The harvesting operation could not be done in a single day because data for each hill had to be taken separately. It took nearly a month to harvest all plants and grains in both cultures. Before the plants in each hill were cut, the average height of the plants was taken. This represented the middle reading of the vertical distance between the tallest and lowest panicle in each hill, measuring from the base of the hill to the first node of the axis of the panicle. The bearing culms were then counted, after which the panicle from the individual bearing culm in each hill was harvested, placed in a paper envelope, and properly labeled. The envelope was then exposed to the sun outside the laboratory for about two weeks to dry, after which a hundred of them selected at random from the control and fertilized group of each culture were obtained. The length of each of the selected panicles was measured from the first basal nodal insertion of the lower rachillae of the panicle to the

tip of the uppermost grain. The filled and unfilled grains for each panicle were counted. The grains were then threshed by hand, cleaned, and placed again in the paper envelope for further study.

The filled grains from the panicles obtained from a hundred hills selected at random from the control and fertilized group of each of the cultures were mixed together. From this mixture, one hundred of the hundred-grain samples were obtained at random. The weight of the hundred-grain sample before and after the hulls had been carefully removed by hand was taken according to the procedure described by Apaoan (1939).

The metrical data (length, breadth, and thickness) of the hulled grains were determined with a micrometer caliper that can easily be read to the hundredth of a millimeter. Altogether, 40,000 hulled grains from each culture were measured.

Chemical analyses. On May 14, 1938, samples of unhulled and hulled grains harvested from the control and fertilized groups derived from both can and field cultures were submitted to the Department of Agricultural Chemistry, for analysis.

RESULTS AND DISCUSSION

Tillering

Time of emergence of tillers. Among the materials examined, it was found that the youngest leaves of the tillers were out of their subtending leaf sheaths on January 2, 1938, exactly thirteen days after transplanting and twenty-four days after seeding. A greater proportion of the plants in either culture did not exhibit marked tiller development until a week later, on January 9, 1938, when the first actual counting was made. Benerjee, Bose, and Mahalanobis (1936), and Juliano (1938) found that tiller formation began a week after transplanting, but in this particular study it was about a week late. However, Ramiah and Narashimhan (1936) stated that transplanted rice plants began active tillering about two weeks after transplanting and were in full swing for three to five weeks, depending on the life duration of the variety.

Table 1 shows the weekly mean increment of tillers per plant in both the can and field plants. In both cultures there was a steady increase in the mean number of tillers per plant until the forty-eighth day or approximately the seventh week after transplanting and fifty-nine days or a little over eight weeks after seeding in the control

can plants. In the case of the fertilized can plants as well as of the control and fertilized field plants, the steady increase in tiller formation lasted a week longer than in the control can plants (fig. 1), so that the "critical stage" in tiller formation was much delayed by fertilization in the can plants and in the field plants, whether fertilized or not. After this critical stage was reached, there was a slight increase or decrease in tiller formation, because of the formation of new late tillers or the death of early or late tillers. The differences of the weekly mean number of tillers per plant in both cultures after February 6, 1938, and February 13, 1938, respectively, were all insignificant.

Mean number of tillers per plant. Table 1 and figure 1 show that in general can plants produced a much larger mean number of tillers per plant than those grown in the field, except the control can plants where a smaller mean number of tillers per plant was obtained when compared with similar plants grown in the field. Juliano (1938) found that in his cultures, can grown plants always produced a larger mean number of tillers per plant than the field grown plants.

Fertilization influenced tiller formation in rice (table 1) and its effect was rather favorable within limits. Table 1 shows that whenever fertilization was resorted to, the mean number of tillers per plant was much increased (fig. 1) and that all differences in their weekly counts were significant in favor of fertilization two weeks after transplanting.

Average height

For one familiar with rice, it is common to observe that plants grown in the field are taller than those grown in cans (Juliano, 1938). In this particular study, this fact was further confirmed (fig. 3). In all cases the differences in the average mean height of the mature plants were significant in favor of the field plants. Fertilization had also a similar influence on the average mean height of the plants whether they were grown in cans (fig. 2) or in the field (table 2).

Booting and flowering

Booting and flowering in both cultures took place at different times. Control can plants booted from March 3 to 6, 1938, while those in the field (control) from March 3 to 14, 1938. In the fertilized can plants, booting was recorded from March 4 to 10, 1938,

while in the fertilized field plants this was noticed from March 14 to 16, 1938. It seems that fertilization somewhat delayed booting in both can and field grown plants, although the difference was only one to three days.

Flowering in the control can plants took place from March 10 to 27, 1938, while in the fertilized ones, from March 11 to 30, 1938.

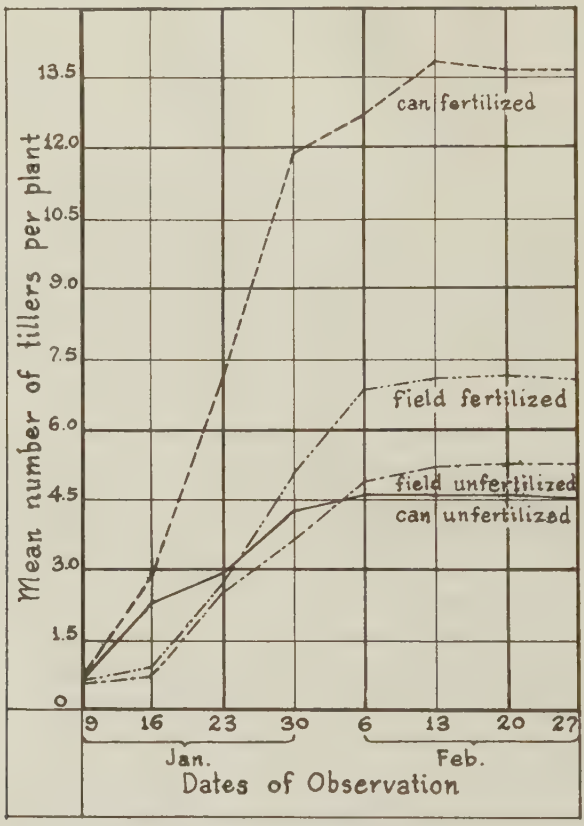

Fig. 1.—Comparative weekly increment of tillers per plant.

Field-grown plants which were fertilized flowered from March 20 to April 1, 1938, while in the control plants, flowering was recorded from March 14 to April 1, 1938. It seems that fertilization also delayed the time of flowering in both cultures. Flowering among fertilized plants was more uniform than among the controls. In general, it took about a week for all the inflorescences in a single

hill to emerge from their leaf sheaths from booting, and another week or so for all of them to finish flowering.

Mean number of bearing culms

In rice growing the mean number of bearing culms per plant is of great importance as this is one of the factors which determines the production of grains. In the present study fertilization had a pronounced influence on the production of mean number of bearing culms per plant in both cultures (fig. 3). All the differences were

Fig. 2. Comparative stand of can grown plants 58 days after seeding. (*a*) fertilized, and (*b*) control. Photographed Feb. 5, 1938, by the Photographic Division, College of Agriculture, University of the Philippines.

significant in favor of fertilization (table 3). When the field and can plants are compared, it is evident that the latter tend to produce a greater mean number of bearing culms than the former (fig. 3), although this does not mean to imply that can grown plants will yield more than their sister plants grown in the field, as other factors have to be taken into account. The difference in the mean number of bearing culms in the control plants was significant, while in the fertilized ones, it was insignificant, although both were in favor of the can grown plants.

Mean length of panicle

Length of the panicle is a good criterion for yield. Table 4 shows that the field grown plants produced much longer panicles than can grown plants (fig. 4). Fertilization affected significantly the mean length of the panicles; that is, the fertilized plants, whether they were planted in cans or in the field, produced much longer pa-

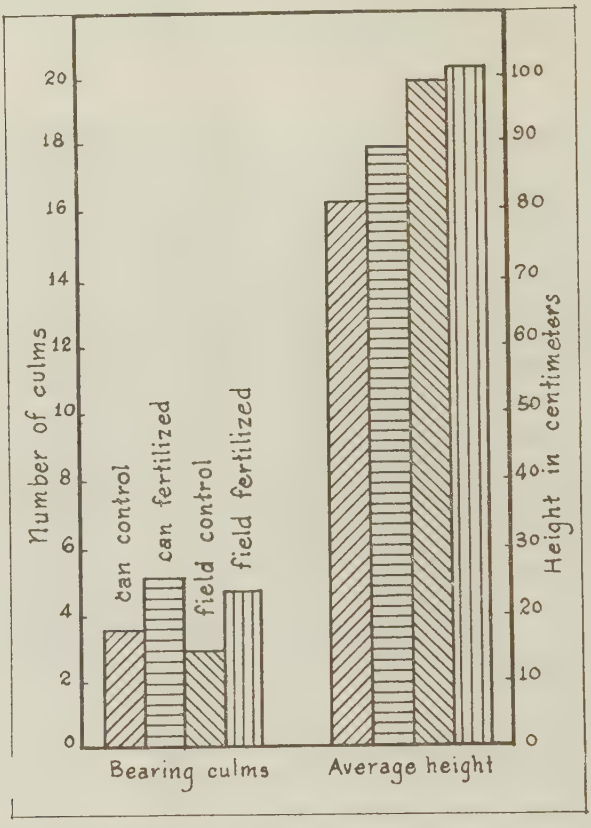

Fig. 3. Characteristics of the plants.

nicles than their sister control plants. The fact that fertilization increases actually the mean length of the panicle speaks highly in favor of such practice for increasing grain production of rice.

Filled and unfilled grains

The number of filled grains per panicle will give one an index of the amount of harvest to be obtained from a stand of rice plants. Production of filled grains, however, is dependent on multifarious

environmental conditions obtaining from the time the inflorescence emerges from the sheath up to the time the grains attain maturity. Table 5 gives the mean number of filled grains per panicle produced from both cultures. The mean number of filled grains per panicle was significantly higher in the field plants (fig. 4) than in cans; that is, panicles produced by plants in the field had a higher mean number of filled grains per panicle than those in cans. Fertilization, whether done in cans or in the field, increased the mean number of filled grains per panicle, a fact that should not be ignored in the culture of rice.

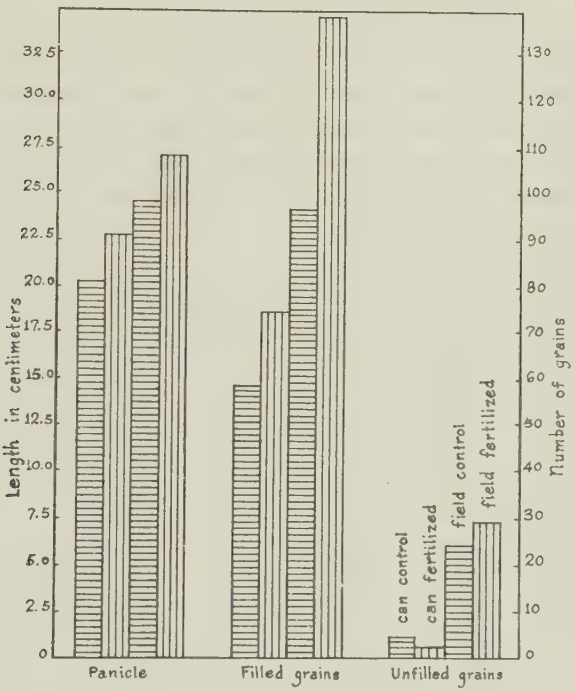

Fig. 4. Relative characteristics of the panicle.

As regards the mean number of unfilled grains (table 5) per panicle, plants grown in the field yielded a significantly higher mean number of unfilled grains than those grown in cans, a fact that was contrary to what had been previously found by the senior author (Juliano, 1938). Fertilization did materially increase the mean number of unfilled grains in the field plants (fig. 4), while it significantly reduced them in plants grown in cans under conditions then obtaining in this experiment.

If the mean number of filled and unfilled grains per panicle can be taken as an index of the size of the panicle, then the results herein presented showed that panicles produced by can grown plants were much smaller than those from field grown plants, a fact that had been found also by Juliano (1938).

Weight of grains

Unhulled grains. The mean weight of a hundred-grain sample from can grown plants was insignificantly lower than that from similar field grown plants (table 6), whether the plants were fertilized or not. On the other hand, fertilization brought about significant increase in the mean weight of a hundred-grain sample from plants grown in cans and in the field over those of the corresponding control cultures. In other words, there was a tendency for the mean weight of the grains to increase after being fertilized or planted in the field. This increase may be due to an increase in the weight of the hull, increase in the size of the grain, or increase in the amount of reserved food in the kernel.

Hulled grains. Tin (1936) also found that ammoniacal fertilizers alone or in combination with other fertilizers caused an increase in the mean whole grain weight. Significant increase of the weight of hulled grains was due only to the addition of two fertilizers, namely, ammonium sulfate and superphosphate. Regarding the effect on the polished rice, which is the most important part of the plant, ammonium sulfate and its combinations produced significant increases. Hull and bran were only affected slightly by the addition of fertilizers. Increase in grain weight caused by fertilization was due mainly to the increase in the endosperm and not to the hull or bran. After each of the hundred-grain sample from both cultures was weighed, the hulls of the grains were carefully removed by hand and then reweighed. Data on the weight of hulled-grain samples are also compared in table 6. It is very clear from the data herein presented that mean weight of the hulled hundred-grain sample from plants grown in the field was insignificantly greater than that of plants grown in cans, a proof that there existed direct correlation between the weight of the unhulled and hulled grains (Apaoan, 1939). Fertilization in all cases increased insignificantly the mean weight of the hulled hundred-grain samples in both cultures. The increase in weight of the sample may be due either to the increase in the size of the hulled grains or to the amount of their reserved food.

Table 6 gives the mean weight of the unhulled and hulled hundred-grain sample; the relative weight of the hull can thus be computed. The difference between the mean weight of the unhulled grains and that of the same sample after the hulls have been removed represents the hull. Results show that the weight of the hull per given sample increased with fertilization from less than twice in the field plants (control - 0.180 ± 0.1731 gram; fertilized - 0.300 ± 2470 gram) to more than five times in the can plants (control - 0.050 ± 0.2157 gram; fertilized - 0.290 ± 0.2261 gram). In other words, the increase in weight of unhulled grains may be due in part to the increase in weight of the hull.

Size of grains

The importance of the size of the grains in the determination of rice varieties has been brought out repeatedly by Juliano (1938, 1939, 1940) and Apaoan (1939). Table 7 gives the results of measurement of the length, breadth, and thickness of the hulled grains from the control and fertilized plants grown both in cans and in the field. In this study a total of 40,000 grains were measured.

Length. Results herein presented revealed that the mean length of the hulled grains obtained from plants grown in cans and in the field did not vary considerably. The mean length of hulled grains from field grown plants was insignificantly more than that from similar can grown plants, whether the plants were fertilized or not. However, if the measurements of the hulled grains obtained from the fertilized and control plants in the same culture are compared, it is apparent that the mean length of the hulled grains from the former is significantly more than that of the latter; that is, fertilization tended somewhat to increase the mean length of the hulled grains, and consequently, perhaps, the size.

Breadth. Tin (1936) stated that paddy grain dimensions are positively affected by the addition of ammonium sulfate in combination with superphosphate and potassium sulfate. Ammonium sulfate alone seemed to have a great effect, though statistically not definite, but the addition of the other slighly effective manures increased the length definitely. In mean breadth the field grown plants produced significantly broader hulled grains than the can grown plants (table 7), whether they were fertilized or not. Can grown plants which received the fertilizer produced significantly broader hulled grains than their sister plants without fertilizer. On the other hand,

field grown plants which were fertilized had insignificantly broader hulled grains than those which were unfertilized. In other words, mean breadth of hulled grains was significantly and favorably affected by fertilization when the plants were grown in cans but insignificantly when grown in the field.

Thickness. In mean thickness of hulled grains, there were found no significant differences between plants grown in cans and in the field and whether the plants were fertilized or not. Fertilization as usual brought out an insignificant increase in mean thickness of hulled grains in can grown plants, while significant increase was noticed in the field plants.

In general, therefore, results herein presented tend to show that fertilization affected significantly the size (length, breadth, and thickness) of hulled grains, although of all characters of the rice plant studied, this was the least affected. This fact further corroborates the claim (Juliano, 1938, 1939, 1940; Apaoan, 1939) that size of grain can be depended upon as a good criterion in the identification of cultivated rices; upon this many keys for such purposes have already been based (Kikkawa, 1912; Graham, 1913; Beale, 1927; Thadani and Dutt, 1928; Sethi and Saxena, 1930; Mitra and Ganguli, 1932; Hector, Sharngapani, Roy, and Chakravarty, 1934; Gustchin, 1934; Ram and Chetty, 1934; Ram and Eckbote, 1936; Piacco, 1936, 1937a, 1937b).

Chemical analyses

Chemical analyses of hulled and unhulled grains harvested from the fertilized and control plants from both cultures (can and field) are shown in table 8. Results of analyses may indicate whether the increase in weight and size of the grains was due to the actual increase in the amount of reserve food in the kernel or not.

Unhulled grains. Grain samples from control can plants contained more fat, carbohydrates, and calories per 100 grams but less moisture, ash, protein, and crude fiber than those from field plants.

Grains from the fertilized can plants contained less fat, ash, and protein but more moisture, crude fiber, carbohydrates, and calories per 100 grams, than those from the field plants. These results seemed natural in view of the fact that plants in the field had unlimited access to nutrients found in the soil, while those grown in cans were limited in their supply.

If grains from the control and fertilized plants in each culture were to be compared, the figures gave divergent results. While the

grains from the control can plants possessed higher fat, ash, and protein content than those from the fertilized can plants, those from the control field plants excelled only in fat, having much less ash and protein than the fertilized ones.

Hulled grains. Chemical analyses of hulled grains gave as surprising figures (table 8) as of unhulled grains. Grains from control can plants had less moisture, fat, ash, and protein, but more of all the other items than did the grains from the field plants. This condition was practically the reverse of that obtaining in unhulled grains. Grains from the fertilized can plants had less moisture and protein content but more fat, ash, crude fiber, carbohydrates, and calories per 100 grams than those from the field plants. In other words, while fertilization tended to increase the nutritional constituents of the grains in one culture, the reverse was found to be true in the other cultures. However, analyses herein presented seemed to indicate that fertilization improved somewhat the nutritional properties of the grains. Inasmuch as chemical properties of the unhulled and hulled grains whether fertilized or otherwise are not constant, its use in classification is not reliable.

SUMMARY AND CONCLUSIONS

1. Fertilization with ammonium sulfate somewhat lengthened the time for the plants to reach their "critical stage" in tiller formation.

2. In general, can grown plants had more tillers than field grown plants; however, fertilization increased significantly the mean number of tillers per plant, whether they were grown in cans or in the field.

3. Plants grown in the field were significantly taller than those grown in cans and fertilization significantly influenced favorably the average mean height of the plants.

4. Booting and flowering seemed to be influenced only by fertilization, which caused a delay.

5. The field grown plants produced less mean number of bearing culms than can grown plants, but fertilization significantly favored the production of more bearing culms per plant.

6. Field plants had longer panicles than can grown plants. Fertilization produced significant increase in length of the panicle.

7. The filled grains per panicle were significantly more numerous in the field plants than in the can grown plants, and much more

so when fertilized. The field plants had also significantly more unfilled grains than can grown plants.

8. In mean weight of hundred-grain sample (unhulled or hulled), the field plants exhibited heavier grains than the can plants. Fertilization also increased the mean weight of the grains and their hulls.

9. Of the three dimensional characters of the hulled grains, length and thickness were the least influenced by environment. Fertilization seemed to increase significantly or not these characters of the hulled grains.

10. The results of chemical analyses of the unhulled and hulled grains from the control and fertilized plants of both cultures were very variable, although fertilization seemed to influence favorably the nutritional value of the grains.

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TABLE 1

Analysis of weekly increment of tillers per plant

DATES	CAN CULTURE					FIELD CULTURE					MEAN DIFFERENCE (CAN AND FIELD)	
	Unfertilized		Fertilized		Mean Difference	Unfertilized		Fertilized		Difference	Unfertilized	Fertilized
	Mean	Standard deviation	Mean	Standard deviation		Mean	Standard deviation	Mean	Standard deviation			
Jan. 9, 1938	0.68 ± 0.0462	0.46167	0.72 ± 0.0521	0.52068	0.04 ± 0.0696	0.52 ± 0.0144	0.1435	0.51 ± 0.0161	0.10105	−0.010 ± 0.0176[a]	0.16 ± 0.0484	0.010 ± 0.0531
Jan. 16, 1938	2.46 ± 0.0462	0.46232	2.89 ± 0.0911	0.91126	0.43 ± 0.1021	0.86 ± 0.0669	0.6690	0.98 ± 0.0969	0.96940	0.080 ± 0.3119	1.60 ± 0.0805	1.91 ± 0.1328
Jan. 23, 1938	2.88 ± 0.0879	0.87870	7.09 ± 0.1370	1.36998	4.21 ± 0.1627	2.46 ± 0.0621	0.6208	2.71 ± 0.0655	0.65458	0.250 ± 0.0902	0.42 ± 0.0999	4.38 ± 0.1518
Jan. 30, 1938	4.25 ± 0.0848	0.84829	11.90 ± 0.2754	2.73404	7.75 ± 0.2852	3.64 ± 0.2098	2.0984	5.05 ± 0.1186	1.18598	1.41 ± 0.2410	0.61 ± 0.2263	6.85 ± 0.2978
Feb. 6, 1938	4.51 ± 0.0785	0.78502	12.63 ± 0.2680	2.67989	8.12 ± 0.2810	4.89 ± 0.1016	1.0161	6.82 ± 0.1129	1.12922	1.93 ± 0.1518	−0.38 ± 0.1281[b]	5.81 ± 0.2908
Feb. 13, 1938	4.51 ± 0.0785	0.78502	13.46 ± 0.2499	2.49881	8.95 ± 0.2619	5.16 ± 0.1270	1.2695	7.05 ± 0.1355	1.35646	1.89 ± 0.1857	−0.65 ± 0.1494[b]	6.41 ± 0.2843
Feb. 20, 1938	4.57 ± 0.0801	0.80082	13.17 ± 0.2424	2.42441	8.60 ± 0.2552	5.25 ± 0.1368	1.3677	7.17 ± 0.1517	1.51720	1.92 ± 0.2042	−0.68 ± 0.1426[b]	6.00 ± 0.2859
Feb. 27, 1938	4.52 ± 0.0779	0.77876	13.16 ± 0.2462	2.46520	8.64 ± 0.2381	5.26 ± 0.1420	1.4201	7.15 ± 0.1558	1.55788	1.89 ± 0.2108	−0.74 ± 0.1619[b]	6.01 ± 0.2913

[a] Fertilized less than the unfertilized; difference insignificant.

[b] Can plants less than the field ones; difference significant.

TABLE 2

Average height of plants per hill in centimeters

ITEMS		UNFERTILIZED	FERTILIZED	DIFFERENCE
Can Culture	Mean	80.8500	89.0500	8.20±0.2135
	Standard error ..	0.1323	0.1676	—
	Standard deviation	1.3234	1.6760	—
Field Culture	Mean	99.3500	109.7500	10.40±0.2904
	Standard error ..	0.1798	0.2280	—
	Standard deviation	1.7980	2.2804	—
Difference (field and can)		18.50±0.2232	20.70±0.2829	—

TABLE 3

Mean number of bearing culms per plant

ITEMS		UNFERTILIZED	FERTILIZED	DIFFERENCE
Can Culture	Mean	5.6200	7.1200	1.50±0.2898
	Standard error ..	0.2448	0.1551	—
	Standard deviation	2.4480	1.5512	—
Field Culture	Mean	5.0000	6.7300	1.73±0.2156
	Standard error ..	0.1259	0.1750	—
	Standard deviation	1.5290	1.7502	—
Difference (field and can)		0.62±0.2753	0.39±0.2336	—

TABLE 4

Mean length of panicle in centimeters

ITEMS		UNFERTILIZED	FERTILIZED	DIFFERENCE
Can Culture	Mean	20.4090	22.8100	2.41±0.2433
	Standard error ..	0.1441	0.1960	—
	Standard deviation	2.0390	1.5900	—
Field Culture	Mean	24.8300	27.2100	2.38±0.2888
	Standard error ..	0.2289	0.1761	—
	Standard deviation	2.2890	1.7690	—
Difference (field and can)		4.43±0.2705	4.40±0.2635	—

TABLE 5

Mean number of filled and unfilled grains per panicle

ITEMS		FILLED GRAINS		
		Unfertilized	Fertilized	Difference
Can Culture	Mean	58.8150	74.8550	16.040±0.2335
	Standard error ..	0.2066	0.1088	—
	Standard deviation	2.9220	2.5200	—
Field Culture	Mean	96.7600	138.9400	42.180±0.5279
	Standard error ..	0.2970	0.4248	—
	Standard deviation	2.9700	4.2480	—
Difference (field and can)		37.945±0.3618	64.085±0.4449	—
		UNFILLED GRAINS		
Can Culture	Mean	4.5900	2.1650	2.425±0.2489
	Standard error ..	0.1972	0.1504	—
	Standard deviation	2.7710	2.1270	—
Field Culture	Mean	23.5200	29.8600	6.340±0.6714
	Standard error ..	0.5077	0.4394	—
	Standard deviation	5.0770	4.3940	—
Difference (field and can)		18.93±0.5446	27.695±0.4644	—

TABLE 6

Mean weight of unhulled and hulled hundred-grain sample in grams

ITEMS		UNHULLED GRAINS		
		Unfertilized	Fertilized	Difference
Can Culture	Mean	2.6950	3.2250	0.53±0.2242
	Standard error ..	0.1508	0.1662	—
	Standard deviation	1.5080	1.6620	—
Field Culture	Mean	2.8650	3.2850	0.42±0.2174
	Standard error ..	0.1175	0.1829	—
	Standard deviation	1.1750	1.8290	—
Difference (field and can)		0.17±0.1912	0.066±0.2471	—
		HULLED GRAINS		
Can Culture	Mean	2.6450	2.9350	0.29±0.2172
	Standard error ..	0.1542	0.1533	—
	Standard deviation	1.5420	1.5329	—
Field Culture	Mean	2.6850	2.9850	0.30±0.2091
	Standard error ..	0.1271	0.1660	—
	Standard deviation	1.2705	1.6602	—
Difference (field and can)		0.04±0.1996	0.05±0.2259	—

TABLE 7

Mean length, breadth, and thickness of hulled grains in millimeters

ITEMS		Unfertilized	Fertilized	Difference
		LENGTH		
Can Culture	Mean	6.2036	6.2678	0.0642±0.0262
	Standard error	0.0182	0.0183	—
	Standard deviation	1.8230	1.8300	—
Field Culture	Mean	6.2160	6.2953	0.0793±0.0265
	Standard error	0.0182	0.0164	—
	Standard deviation	1.8150	1.6370	—
Difference (field and can)		0.0124±0.0257	0.0275±0.0249	—
		BREADTH		
Can Culture	Mean	2.2771	2.2224	0.0547±0.0125
	Standard error	0.0085	0.0092	—
	Standard deviation	0.8460	0.9230	—
Field Culture	Mean	2.4029	2.4039	0.0010±0.0127
	Standard error	0.0091	0.0088	—
	Standard deviation	0.9070	0.8780	—
Difference (field and can)		0.1258±0.0129	0.1815±0.0128	—
		THICKNESS		
Can Culture	Mean	1.5672	1.5845	0.0173±0.0144
	Standard error	0.0103	0.0100	—
	Standard deviation	1.0330	1.0020	—
Field Culture	Mean	1.5606	1.5916	0.0310±0.0151
	Standard error	0.0104	0.0109	—
	Standard deviation	1.0430	1.0890	—
Difference (field and can)		−0.0066 ±0.0146	0.0071±0.0148	—

TABLE 8

Chemical analyses of the unhulled and hulled grains [a]

ITEMS	UNHULLED GRAINS				HULLED GRAINS			
	Can culture		Field culture		Can culture		Field culture	
	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized
Moisture	12.69	12.86	12.75	13.04	12.24	12.17	13.24	13.62
Fats (ether extract)	1.07	0.63	1.06	0.68	1.25	1.90	1.40	1.28
Ash	7.64	7.21	8.12	8.62	1.42	1.62	1.51	1.43
Protein (N × 6.25)	5.85	5.41	6.16	8.60	6.91	6.81	7.85	8.85
Crude fiber	10.86	11.46	10.94	10.53	0.79	1.02	0.70	0.67
Carbohydrate (N.F.E.) [b]	61.89	62.43	60.97	58.53	77.39	76.48	75.30	74.15
Calories per 100 grams [b]	288.00	284.00	285.00	282.00	357.00	359.00	354.00	352.00

[a] Analyses made by the Department of Agricultural Chemistry, College of Agriculture. Figures represent average of two samples.

[b] Computed.

FLUCTUATION OF BODY TEMPERATURE IN THE JERSEY AND HOLSTEIN-FRIESIAN BREEDS OF DAIRY CATTLE IN THE PHILIPPINES [1]

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AND

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In their study on the four-hourly fluctuation throughout the day of the body temperature of Indian Nellore cattle in the Philippines, Manresa and Gomez (1937) reported that the highest temperature occurred between two and six o'clock in the afternoon. This coincided with the hottest hours of the day. Manresa and Falcon (1939) found the average body temperature of Nellore cattle during the hottest part of the year, from March to July, to be significantly higher than the average body temperature during the cooler part of the year, from August to February. The correlations between atmospheric and body temperature in Nellores have ranged from 0.337 $\pm$ 0.033 to 0.476 $\pm$ 0.032 (Manresa and Falcon [1939], Manresa, Gomez, and Santos [1939]). On the basis of these findings the suggestion was made that the problem involved in the acclimatization of the various breeds of cattle, especially those originating from the temperate countries, should be studied in the light of their adaptability to the temperature of the air prevailing in given localities.

Early in October, 1939, the senior author concluded an arrangement for the taking of body temperature of the Jersey and Holstein-Friesian breeds of dairy cattle through Mr. Pedro P. Erce, manager of a large dairy farm in Bulacan where these are the principal breeds of dairy cattle used. The farm is situated about 500 feet (152.4 meters) above sea level on a large estate only 29 kilometers from the city of Manila (Hermosura)[2]. Rectal temperature readings were taken by the junior author from fourteen Jersey and seventeen Holstein-Friesian milking cows beginning on October 24, 1939. These readings were made between 6:00 and 8:00 o'clock in the morning,

[1] Experiment Station contribution No. 1381. Read before the Los Baños Biological Club, July 25, 1940.

[2] HERMOSURA, SEVERINO. A survey of dairy farms in the Philippines. (Thesis presented for graduation with the degree of Bachelor of Science in Agriculture from the College of Agriculture. 1939. Unpublished.)

and again between 2:00 and 4:00 o'clock in the afternoon. In every case the thermometers were kept in the rectum for not less than three minutes.

It was the plan to carry the work through one complete year. However, after the work had gone on uninterruptedly for three months, it became necessary to abandon the taking of morning temperatures owing to pressure of work; and on June 1, 1940, the work had to be entirely discontinued. In view of the fact that there is very little likelihood in the immediate future of getting further data on body temperature from as large a number of cattle originating from temperate countries because the raising of these animals in the Philippines has been beset with great difficulties, it seems advisable to report the material on hand. The data obtained comprise 3,416 rectal temperature readings from the Jersey and 4,267 from the Holstein-Friesian cattle.

Table 1 shows that the body temperature of the Jersey cattle between 6:00 and 8:00 a. m. ranged from 37.1 to 41.0°C., with the mean at 38.46 ± 0.013°C. The afternoon temperature of the same breed taken between 2:00 and 4:00 p. m. ranged from 37.5 to 41.0°C., with the mean at 38.80 ± 0.005°C. (table 2). In the Holstein-Friesian cattle the range of the morning temperature was from 37.5 to 40.6°C., with the mean at 38.69 ± 0.013°C. (table 3). The afternoon temperature of this breed (table 4) ranged from 37.5 to 41.4°C., with the mean at 39.12 ± 0.006°C.

In both Jersey and Holstein-Friesian cattle there was slightly more variability in the morning temperatures than in the afternoon. The coefficients of variation of these temperatures were 1.24 ± 0.024 per cent in the morning as against 1.05 ± 0.010 per cent in the afternoon in the Jersey breed of cattle and 1.36 ± 0.023 per cent as against 1.09 ± 0.008 per cent, respectively, in the Holstein-Friesian. The differences between the morning and afternoon temperatures in each breed were significant. Thus, in the Jersey the afternoon temperature was higher than the morning temperature by 0.34 ± 0.014°C., the difference being twenty-five times its probable error. In the Holstein-Friesian, the afternoon temperature was higher than the morning temperature by 0.45 ± 0.015°C. These findings are in agreement with those reported by Manresa and Gomez (1937) and Manresa and Falcon (1939) for the Indian Nellore breed of cattle raised in the College of Agriculture, wherein the highest body temperatures coincided with the time of the day and months of the year characterized by high atmospheric temperatures.

When afternoon temperatures of the Jersey and Holstein-Friesian cattle were compared, it was found that the Holstein-Friesian consistently showed higher body temperature than the Jersey. In these comparisons Snedecor's statistical method (1938) designed for group comparisons was used in view of the fact that the experiment was carried on for a period of only eight months instead of one complete year. This method enables one to determine whether the sample for each set and from the different animals was sufficient for the test of significance. Since Snedecor's method for group comparisons requires random pairing of individuals, the twelve Jersey cows with complete and uninterrupted temperature data from October, 1939, to June, 1940, were matched with the first twelve of the seventeen Holstein-Friesian cows with complete and uninterrupted temperature data during the same period. A summary of these data taken between 2:00 and 4:00 o'clock p.m. is given in the following tabulation:

Summary of body temperature data of Jersey and Holstein-Friesian cows taken between 2:00 and 4:00 p. m. from October, 1939, to June, 1940

BREEDS OF CATTLE	NUMBER OF DETERMINATIONS	DEGREES OF FREEDOM	MEAN °C.	SUM OF SQUARES OF DEVIATIONS FROM THE MEAN
Jersey	217	11	38.85	0.3512
Holstein-Friesian	217	11	39.12	1.7242
		Sum = 22	d = 0.27	Sum = 2.0755

$$\text{Pooled variance} = V = \frac{\text{Sum of squares of deviations}}{\text{Sum of degrees of freedom}} = \frac{2.0755}{22} = 0.0943$$

$$\text{Standard deviation} = s_d = \sqrt{0.0943} = 0.30\,^{\circ}\text{C.}$$

$$\text{Standard error} = s_e = \sqrt{2V/n} = 0.029\,^{\circ}\text{C.}$$

$$\text{Value of "t"} = \frac{d}{s_e} = \frac{0.27}{0.029} = 9.31$$

It was found that the difference, 0.27°C., in the body temperature between the Jersey and Holstein-Friesian from 2:00 to 4:00 p. m. with standard deviation, s_d = 0.30°C., was sufficient for significance; and that at the level of 5 per cent, twelve rectal temperature readings from each of the twelve animals in each group would have satisfied the requirement for the test of significance, whereas twenty-five would

have been sufficient for high significance. Actually 217 readings were made. It would not be necessary, then, to carry on the experiments for one year. For the test of significance in individual comparisons of the difference, 0.27°, between the afternoon temperatures of the Holstein-Friesian and Jersey, with standard error 0.029, the value of "t" is found to be 9.3. At the 5 per cent level, the value of "t" for eleven degrees of freedom given in Fisher's table is 2.201. Evidently then, the Holstein had significantly higher temperature than the Jersey.

Smith (1916) cites Wooldridge, who gives the range of fluctuation of rectal temperature for normal dairy cattle in the United States, breed not mentioned, from 38.0 to 39.3°C., with the mean at 38.6°C. Dukes (1937) states that Hewitt found the average for beef cows in the United States to be 38.3°C., the range being from 36.7 to 39.1°C. These ranges are much narrower than those of the Jersey and Holstein-Friesian cattle reared in the Philippines. In view of the higher mean monthly range of atmospheric temperature in the Philippines compared to that prevailing in the United States and in the homes of origin of these breeds of cattle, a corresponding rise in body temperature is expected in Jersey and Holstein-Friesian cattle reared in the Philippines. It was found, however, that while the mean of the morning and afternoon temperatures of the Jersey reared in the Philippines is the same as that reported for dairy cattle in the United States, that of the Holstein-Friesian reared in the Philippines is very much higher than the average for the dairy cattle in the United States. The great difference in size between the Jersey and Holstein cattle was the factor named by Davidson (1927) to account for the unsuitability of the Holstein-Friesian in the southern United States. The Holstein, being bigger feeders than the Jersey according to Davidson, normally function on a higher plane of nutrition and, hence, are more sensitive to high temperatures and high humidities. They are also not as well equipped for the radiation of heat from their bodies as are the Jersey owing to their greater size.

In their histological studies on the structure of the hide of buffalo and of Zebu and Holstein-Friesian cattle in relation to the problem of tropical acclimatization, Yamane and Ono (1936) concluded that, from the point of view of heat regulation through the surface of the skin, the Zebu is better adapted than the Holstein. They found that the skin of the Holstein is characterized by weak development of the epidermis but has a relatively well developed papillary layer.

In spite of rapid growth of their bodies, the sebaceous and sweat glands in Holstein cattle are decidedly smaller in size and less in number per unit area than in Zebu.

Available data on rectal temperature of Indian Nellore cattle taken between 2:00 and 4:00 p. m. for one complete year in the College of Agriculture show the mean body temperature at these hours to be 38.82 ± 0.022°C. This temperature is not significantly different from the mean body temperature of the Jersey at 2:00 to 4:00 p. m. The temperature of the Holstein-Friesian, however, at these hours is significantly higher than that of the Indian Nellore. Those who have sought a physical explanation for the suitability of the Indian Nellore cattle to the tropics upon the basis of radiation of heat from the surface of the body (Kelley, 1932, and Littlewood, 1936) have pointed out the following characters in the prevailing breeds of Zebu cattle which they believe to facilitate greatly the rapid loss of heat from the surface of the skin: hump at withers, long and drooping ears, very loose dewlap, generally thinner skin of prepuce and umbilicus, great height at the shoulder where hump is attached, relatively narrow hip bones, short and smooth hair coat, and black skin covered by white, grey, or red hair. In these characteristics the Holstein-Friesian are exactly the opposite.

Yamane and Ono (1936) found the buffalo hide to be structurally unsuitable for tropical acclimatization. This fact notwithstanding, the buffalo can stand tropical climate for the reason that this animal has become adapted to the habit of burying its body under water during the hotter part of the day or even during the night whenever the air temperature is warm. If the high body temperature were the only factor which incapacitates the Holstein-Friesian from being generally acclimated to the Philippines, it would seem quite possible to remedy this situation by some artificial means. The system of dairy management adopted by the Singapore Dairy Farm (Villegas, 1939) appears to have largely avoided the unfavorable effects of external heat in Singapore. In this system of management the animals are kept loose in herds inside the barns at all times except in the morning from 5:30 to 10:30 o'clock. If weather permits, the animals are placed in the pasture at night. To make the barns cool, nipa is used for roofing. The sides of the barns are kept open and, to enhance greater ventilation, air is pumped in through large galvanized iron tubes. This keeps the atmosphere in the barn constantly fresh and comfortable.

A further step in the same direction has been successfully taken in Singapore by placing heavy milking Holstein-Friesian cows in air-conditioned dairy barns. The greatest drawback to such a method of dairy cattle management lies in the cost of production, which necessarily must be high, thus limiting the business to relatively fewer men who can rely on frequently imported stock for the greater bulk of their milking animals since the average cattle raiser who must depend on natural means for his success in raising farm animals is ruled out of the game.

One evidence that environment temperature is not the only factor involved in the hazards of raising cattle of temperate climate origin in the Philippines under natural conditions is found in the Jersey cattle whose body temperature is not significantly different from that of the Indian Nellore raised in the Philippines. Nevertheless, the raising of Jerseys in the Philippines has not been generally successful. Until a breed of dairy cattle suitable for raising under natural conditions in the country has been evolved, lasting progress towards building up the dairy industry of this country cannot be attained.

SUMMARY AND CONCLUSIONS

1. The means of the rectal temperature of Jersey cattle reared in the Philippines were 38.46 ± 0.013°C. in the morning and 38.80 ± 0.005 °C. in the afternoon. In the Holstein-Friesian, they were 38.69 ± 0.013°C. in the morning and 39.12 ± 0.006°C. in the afternoon.

2. In both breeds of cattle the afternoon temperatures were significantly higher than the morning temperatures.

3. The afternoon temperature of the Jersey was not significantly different from the afternoon temperature of the Indian Nellore raised in the Philippines. But the afternoon temperature of the Holstein-Friesian was significantly higher than the corresponding temperature of the Jersey and Indian Nellore. The average daily temperature of the Holstein-Friesian was higher than the average temperature reported for dairy cattle in the United States.

4. If the apparently feverish condition were the only factor which had incapacitated the Holstein-Friesian from being acclimated in the Philippines, it would seem quite possible to remedy this difficulty by artificial means. It is not likely, however, to have been the only factor involved in the problem. The body temperature of the Jer-

sey reared in the Philippines was not significantly different from that of the Indian Nellore; nevertheless, the raising of the Jersey in this country has not been uniformly successful.

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TABLE 1

Frequency distribution of rectal temperature of Jersey cattle taken between 6:00 and 8:00 a. m. during the months of October, November, and December, 1939

RANGE OF TEMPERATURE DEGREES C.	VALUE (V)	F	FV	D	FD^2
37.1–37.4	37.2	1	37.2	1.26	1,5876
37.5–37.8	37.6	21	789.6	0.86	15.5316
37.9–38.2	38.0	138	5,244.0	0.46	29.2006
38.3–38.6	38.4	247	9,484.8	0.06	0.8892
38.7–39.0	38.8	110	4,268.0	0.34	12.7160
39.1–39.4	39.2	41	1,607.2	0.74	22.4516
39.5–39.8	39.6	22	871.2	1.14	28.5912
39.9–40.2	40.0	4	160.0	1.54	9.4864
40.3–40.6	40.4	2	80.8	1.94	7.5272
40.7–41.0	40.8	1	40.8	2.34	5.4756
41.1–41.4	41.2				
Totals		587	22,585.6		133.4570

Mean = 38.46 ± 0.013 °C.
ζ = 0.48 ± 0 009 °C.
c. v. = 1.24 ± 0.24%

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TABLE 2

Frequency distribution of rectal temperature of Jersey cattle taken between 2:00 and 4:00 p. m. for a period of eight months from October, 1939, to June, 1940

RANGE OF TEMPERATURE DEGREES C.	VALUE (V)	F	FV	D	FD^2
37.1–37.4	37.2				
37.5–37.8	37.6	1	37.6	1.2	1.44
37.9–38.2	38.0	26	988.0	0.8	16.64
38.3–38.6	38.4	901	34,598.4	0.4	144.16
38.7–39.0	38.8	1228	47,646.4	—	—
39.1–39.4	39.2	478	18,737.6	0.4	76.48
39.5–39.8	39.6	113	4,474.8	0.8	72.32
39.9–40.2	40.0	54	2,160.0	1.2	77.76
40.3–40.6	40.4	19	767.6	1.6	48.64
40.7–41.0	40.8	9	367.2	2.0	36.00
41.1–41.4	41.2				
Totals		2829	109.777.6		473.44

Mean = 38.80 ± 0.005 °C.
ζ = 0.41 ± 0.004 °C.
c. v. = 1.05 ± 0.015%

TABLE 3

Frequency distribution of rectal temperature of Holstein-Friesian cattle taken between 6:00 and 8:00 a. m. during the months of October, November, and December, 1939

RANGE OF TEMPERATURE DEGREES C.	VALUE (V)	F	FV	D	FD^2
37.1–37.4	37.2				
37.5–37.8	37.6	22	827.2	1.09	26.1382
37.9–38.2	38.0	103	3,914.0	0.69	49.0383
38.3–38.6	38.4	210	8,064.0	0.29	17.6610
38.7–39.0	38.8	217	8,419.6	0.11	2.6257
39.1–39.4	39.2	108	4,233.6	0.51	28.0908
39.5–39.8	39.6	47	1,861.2	0.91	38.9207
39.9–40.2	40.0	22	880.0	1.31	37.7542
40.3–40.6	40.4	2	80.0	1.71	5.8482
40.7–41.0	40.8				
41.1–41.4	41.2				
Totals		731	28.279.6		206.0771

Mean = 38.69 ± 0.013 °C.
ζ = 0.53 ± 0.009 °C.
c. v. = 1.36 ± 0.023%

TABLE 4

Frequency distribution of rectal temperature of Holstein-Friesian cattle taken between 2:00 and 4:00 p. m. for a period of eight months from October, 1939, to June, 1940

RANGE OF TEMPERATURE DEGREES C.	VALUE (V)	F	FV	D	FD²
37.1–37.4	37.2				
37.5–37.8	37.6	2	75.2	1.524	4.6451
37.9–38.2	38.0	18	684.0	1.124	22.7408
38.3–38.6	38.4	485	18,624.0	0.724	254.2253
38.7–39.0	38.8	1,145	44,426.0	0.324	120.1975
39.1–39.4	39.2	964	37,788.8	0.076	5.5680
39.5–39.8	39.6	489	19,364.4	0.476	110.7956
39.9–40.2	40.0	308	12,320.0	0.876	236.3518
40.3–40.6	40.4	100	4,040.0	1.276	162.8176
40.7–41.0	40.8	23	938.4	1.676	64.6064
41.1–41.4	41.2	2	82.4	2.076	8.6194
Totals		3,536	138,343.2		990.5675

Mean = 39.12 ± 0.006 °C.
ζ = 0.53 ± 0.004 °C.
c. v. = 1.09 ± 0.008%

COLLEGE AND ALUMNI NOTES

Dr. Roman Bersamin, formerly resident physician and assistant director of physical education at the College of Arts and Sciences in Baguio, was transferred to the College to take charge of the infirmary effective June 3.

Mrs. Guadalupe Fores Ganzon, formerly supervisor of social sciences in the Manila high schools, was appointed instructor in the Department of Agricultural Education effective June 17. Mrs. Ganzon is an alumna of the University where she obtained the degrees of Bachelor of Science in Education, *summa cum laude*, and Master of Arts.

Dr. F. M. Fronda, Associate Professor of Poultry Husbandry, arrived on July 18 aboard the SS "Kamo Maru" from an extended visit to Victoria, New South Wales, Queensland, Australia, and New Zealand. While there, Dr. Fronda made an extensive study of the poultry and livestock industries and at the same time took opportunity to invite research centers to the forthcoming Seventh Pacific Science Congress to be held in Manila. He brought home one hundred hatching eggs from Australian layers.

Dr. Eulalio P. Baltazar returned to the campus on June 6 from a study of cotton growing in Lagao Settlement, Koronadal Valley, Cotabato, Mindanao. The trip was made at the request of the National Land Settlement Administration.

The officers of the College student body for the first semester are: president, Pastor Manalo; vice-president, Hipolito Custodia; secretary, Francisco Rentutar; treasurer, Nelia Teodoro; representatives to the U. P. Student Council, Luciano Lactao and Glicerio Garcia; and representative to the Board of Management of the Philippine Collegian, Bruno Santos. Dr. Valente Villegas is the adviser.

Dr. Sam Higginbottom, founder and head of the Agricultural Institute, Allahabad, India, was guest speaker at a convention held at the College on July 31. He spoke about India, its people, and its problems.

The following papers were read and discussed before the regular meeting of the Los Baños Biological Club on July 25:

Dr. M. Manresa and Mr. P. P. Erce. The fluctuation of body temperature in Jersey and Holstein-Friesian breeds of cattle in the Philippines.

Dr. N. B. Mendiola and Mr. T. Mercado. Introduction and trial culture and ambon bananas in the College of Agriculture.

Dr. Francisco O. Santos, professor and head of the Department of Agricultural Chemistry, gave a public lecture at the College of Arts and Sciences, Baguio, on August 10. His subject was "Foods for Filipinos".

Mr. Santiago R. Cruz, U. P. fellow taking graduate work in agricultural engineering at Cornell University, has been exempted from the qualifying examination for Ph.D.

Mr. Martin S. Celino of the Department of Plant Pathology qualified for the degree of Master of Science in Plant Pathology by passing the oral examination on August 6. His thesis is "Mosaic of abacá (*Musa textilis* Née)".

Mr. Leandro Garcia was appointed instructor in agricultural engineering effective July 11. Mr. Garcia holds the degree of Bachelor of Science in Civil Engineering from the University of the Philippines.

A portrait of President Bienvenido M. Gonzalez was donated to the College by the students at a special program held on August 8.

Professor Kazuo Ogasahara of the Taihoku Imperial University visited the College on August 20 and 21. Professor Ogasahara has been commissioned by his University to study the probable bearing of Philippine climate on the Oriental migratory locust and the relationship thereof with the swarms which invade Formosa and other neighboring Japanese owned islands from time to time. He discussed the various aspects of this question with workers on this subject in the College.

THE EXPERIMENT STATION

LIST OF AVAILABLE CIRCULARS

Circular No. 2.—Bud Rot of Coconut (Revised, June, 1934) - - - *By G. O. Ocfemia*

Circular No. 3.—Experimental Errors and Application of the Probable Error to and the Interpretation of Experimental Results - - - - - - - - - - - - - *By Nemesio B. Mendiola* (Published as Chapter IV in *A Manual of Plant Breeding for the Tropics*, 1926, also sold by THE PHILIPPINE AGRICULTURIST at ₱3.25, paper bound, and ₱5.25, cloth bound, in the Philippines, and ₱3.50 and ₱5.50 elsewhere, postpaid.)

Circular No. 5.—Poultry Raising Made Easy (Revised) - - - - *By F. M. Fronda*

Circular No. 7.—How to Produce New Varieties of Gumamela (Hibiscus) - - - - - - - - - - - - *By Nemesio B. Mendiola*

Circular No. 8.—Horse Breeding in the Philippines - - - - - *By Valente Villegas*

Circular No. 9.—Fences for Farm Animals - *By B. M. Gonzalez and J. P. Esguerra*

Circular No. 10.—Practical Directions for Coffee Planting - - - *By Pedro A. David*

Circular No. 11.—The New College Copra Drier—Prepared in *Department of Agricultural Chemistry* with cooperation of *Department of Agronomy and Extension.* (Revised by *Moises M. Kalaw*).

Circular No. 14.—Beriberi: Its Causes and Prevention - - - - - *By F. O. Santos*

Circular No. 15.—Cattle Raising under Philippine Conditions - - *By Valente Villegas*

Circular No. 16.—A Simple Farm Record for the Farmer - - *By Francisco M. Sacay*

Circular No. 17.—College Trapnest - - - - - - - *By F. M. Fronda and P. S. Paje*

Circular No. 18.—Surveying for Area with a Surveyor's Staff - *By Alexander Gordon*

Circular No. 19.—A Pageant: Illustrating the History and Growth of the College of Agriculture - - - - - - - - *By Anne F. Cole*

Circular No. 20.—Amount of Nutrients in Philippine Food Materials - - - - - - - - *By F. O. Santos and S. J. Ascalon*

Circular No. 21.—Cassava Growing and Cassava Starch Manufacture - - - - - - - - - - *By Nemesio B. Mendiola*

Circular No. 22.—Goat Raising - - - - - - - - - - - - - *By Valente Villegas*

Circular No. 23.—Curing Pork and Making Sausage for Home Use - - - - - - - - - - - - - - *By Mariano Mondoñedo*

Circular No. 24.—Construction and Operation of Silos in the College of Agriculture - - - - - - - - - - - - *By José P. Esguerra*

Circular No. 26.—Training Cattle and Carabaos for Work - - - *By Valente Villegas*

Circular No. 27.—Bunchy-top of Abacá - - - - - - - - - - - *By G. O. Ocfemia*

Circular No. 28.—Cotton Culture - - - - - - - - - - - - *By Eulalio P. Baltazar*

Circular No. 29.—Collegiate Education in Agriculture - - - - *By L. B. Uichanco*

Circular No. 30.—What Should Filipino Ornamental Gardens and Ornamental Plants Be? - - - - - - - - - *By N. B. Mendiola*

Circular No. 31.—How to Slaughter and Dress Farm Animals - - - - - - - *By Valente Villegas and M. Mondoñedo*

The Circulars Nos. 1, 3, 4, 6, 12, 13, and 25 are out of print.

The price of the Circulars listed above is 10 centavos each except Nos. 5, 10, 16, 21, and 30 the price of which is 20 centavos.

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